JUL 25 1952
B 367757

VOLUME 10 NUMBER 7

NUTRITION REVIEWS

THE LIBRARY OF CONGRESS SERIAL RECORD
AUG 4 1952
DEPT

July 1952

Special Article
Fat Metabolism in the Early 1900's 193
Clinical Nutrition
Effect of Surgery on Plasma Levels of Essential Amino Acids 195
The Intestinal Flora of Infants 198
Restriction of Food Intake and Health 199
Nutrient Absorption Following Intestinal Resection 202
Gastrointestinal X-Ray Studies in Thiamine Deficiency 204
Feeding Problems During Flight 205
Animal Nutrition
The Source of Lysine, Tyrosine and Phosphorus for Casein Synthesis 206
Niacin and Tryptophan in Feline Nutrition 207
Experimental Nutrition
The Action of Thyroid, Choline and Inositol on Tissue Cholesterol and Lipids 208
Influence of Route of Administration and of Sex on Serum Iron Levels 210
Aureomycin, Dietary Proteins and the Growth of Mice 211
Enzymatic Conversion of Phenylalanine to Tyrosine 212
Insulin and Cocarboxylase in Diabetes 214
Relation Between Folacin and Tissue Ascorbic Acid 216
Photosynthesis and Coenzymes 218
The Utilization of Vitamin B_{12} 219
Pantothenic Acid Deficiency and Adrenal Insufficiency 221
Notes
Letter to the Editor 223
Recent Nutrition Book 223
Clinical Nutrition Briefs 224

Published Monthly by

THE NUTRITION FOUNDATION, INC.

CHRYSLER BUILDING NEW YORK 17, N. Y.

COPYRIGHT, 1952, NUTRITION FOUNDATION INC., NEW YORK, U. S. A.

Editor

FREDRICK J. STARE, PH.D., M.D., Professor of Nutrition, Schools of Medicine and Public Health, Harvard University; Associate in Medicine, Peter Bent Brigham Hospital, Boston. Editorial Office, 695 Huntington Ave., Boston, Mass.

Assistant Editor

CHRISTINE S. WILSON, 695 Huntington Ave., Boston

Associate Editors

JOSEF BROŽEK, PH.D., University of Minnesota, Stadium South Tower, Minneapolis
CHARLES S. DAVIDSON, M.D., Thorndike Memorial Laboratory, Boston City Hospital
R. W. ENGEL, PH.D., Alabama Polytechnic Institute, Department of Animal Husbandry and Nutrition, Auburn
STANLEY M. LEVENSON, M.D., Army Medical Nutrition Laboratory, Chicago, and Department of Surgery, Medical College of Virginia, Richmond
JEAN MAYER, PH.D., D.Sc., Department of Nutrition, Harvard School of Public Health, Boston
ROBERT E. SHANK, M.D., School of Medicine, Washington University, St. Louis
JAMES H. SHAW, PH.D., Schools of Dental Medicine and Public Health, Harvard University, Boston
ARTHUR H. SMITH, PH.D., Wayne University College of Medicine, Detroit
ELMER H. STOTZ, PH.D., Department of Biochemistry, School of Medicine, University of Rochester, Rochester
CALVIN W. WOODRUFF, M.D., School of Medicine, Vanderbilt University, Nashville

Editorial Committee

REGINALD M. ATWATER, M.D., American Public Health Assn.
GEORGE R. COWGILL, PH.D., Yale University
CONRAD A. ELVEHJEM, PH.D., University of Wisconsin
WENDELL H. GRIFFITH, PH.D., University of California
JAMES S. McLESTER, M.D., University of Alabama
HENRY C. SHERMAN, PH.D., Columbia University
HAZEL STIEBELING, PH.D., Bureau of Human Nutrition & Home Economics
LEROY VORIS, PH.D., National Research Council
RUSSELL M. WILDER, M.D., National Institutes of Health
JAMES R. WILSON, M.D., American Medical Association
JOHN B. YOUMANS, M.D., Vanderbilt University

Subscriptions should be sent to The Nutrition Foundation, Inc., Chrysler Building, New York 17, N. Y. Rate $4.00 per year in the United States and Canada; $4.50 in other countries.

Note: Items may be quoted from NUTRITION REVIEWS without special permission. If quotations are made, the editors ask only that credit be given to NUTRITION REVIEWS and that they be made in such a way that the original meaning of the review is preserved.

JUL 25 1952

NUTRITION REVIEWS

VOL. 10 JULY 1952 No. 7

FAT METABOLISM IN THE EARLY 1900'S

As late as 1924, E. F. Armstrong and J. Allan (*J. Soc. Chem. Ind.* **43,** *207 (1924)*) in an address to the Society of Chemical Industry made the statement that no other province of chemistry had been so little studied in recent years as that of the fats. The same might be said of their biochemistry, but the situation improved steadily and the interest in the lipids has continued to increase up to the present time.

The main reason for early lack of interest was the physical condition of the material, the difficulty of obtaining the compounds in pure form by crystallization from the mixtures found in plant and animal tissues, and the lack of pure compounds with which to compare them. An interesting illustration of the difficulties met with in working on the lipids was the controversy lasting many years as to whether protagon, a constituent of brain, was a pure substance or a mixture. This question occupied the minds of such men as Liebreich, Kossel, Thierfelder and later in this country of Levene and Gies. Protagon was finally shown to be a mixture of sphingomyelin, cerebrosides and probably some other phospholipids.

The same question applied to a lesser degree to their more important constituents, the fatty acids. However, some progress was being made in this country. O. Folin and A. H. Wentworth (*J. Biol. Chem.* **7,** *421 (1910)*) had overcome the difficulty of titrimetric determination of the fatty acids, due to the hydrolysis of their compounds in water, by making the titrations in solvents in which water was absent or in low concentration.

This period was interesting for the development of Knoop's idea of the beta-oxidation of the fatty acids and the finding that beta-oxybutyric and acetoacetic acids were stages in the metabolism of fats. These substances do not normally appear in the organism or in the urine in more than traces, but when much fat is being metabolized, as in starvation or severe diabetes where most of the energy needs of the organism are supplied by fat, these compounds appear, sometimes in large amounts. Dakin did much work in this country on the development of the beta-oxidation theory. The contributions of G. Lusk (*J. Biol. Chem.* **13,** *27 (1912)*) on the specific dynamic action of fat and the work of L. B. Mendel and A. L. Daniels (*Ibid.* **13,** *71 (1912–1913)*) on the behavior of the fat-soluble dyes belong in this period. Mendel and Daniels showed that the dyes followed either fat into the thoracic duct or bile into the portal system, but did not cross the placenta or the kidney filter.

Interest in the process of fat absorption from the intestine produced considerable work in this country and abroad. It seemed strange that fat should be completely hydrolyzed in the intestine only to be at once resynthesized into fat before entry into the circulation. The materials used in testing this question were ethyl esters of the fatty acids (O. Frank, *Z. f. Biol.* **36,** *568 (1898)*), esters of the fatty acids with certain carbohydrates (W. R. Bloor, *J. Biol. Chem.* **11,** *141, 421, 429 (1912)*), and emulsified mineral oil and lanolin, none of which could be found in the chyle. The mineral oil and lanolin had not left the intestine, but the esters were absorbed. As opposing evidence H. C. Bradley and H. S. Gasser (*Ibid.* **11,** *xx (1912)*) found that when mineral oil was dissolved in olive oil and the whole well emulsified, absorption of the mineral oil did take place and the proportion absorbed in the mixture

was the same as in the original mixture. The victory apparently rested with the side favoring splitting before absorption and this assumption has been held almost to the present day. It was shown that conditions for complete splitting were present in the intestine; namely, fat delivered to it in small portions, and an excess of lipase and emulsifying agents.

The question, however, was not entirely settled and it is not surprising that it came up again. J. Mellanby (*Proc. Physiol. Soc., J. Physiol.* **64,** *v* (*1927*)) found that emulsified fat was absorbed from intestinal loops and the same has been shown very recently by M. Shoshkes, R. P. Geyer, and F. J. Stare (*J. Lab. Clin. Med.* **35,** *968* (*1950*)), who reported that mineral oil if homogenized with water till the particles were submicroscopic in size, was absorbed from intestinal loops. The great part of the evidence for "particulate" absorption has been furnished by Frazer and his associates in England in the last few years (A. C. Frazer and H. G. Sammons, *Biochem. J.* **39,** *122* (*1945*)), and although much of his work could not be repeated in this country by B. Bloom *et al.* (*J. Biol. Chem.* **184,** *1* (*1950*)) and H. C. Tidwell (*Ibid.* **182,** *405* (*1950*)), the balance of evidence seems to be with him in his assumption that there are two paths of absorption, as unsplit particles directly into the thoracic duct, and as split fat either into the portal system or into the thoracic duct with resynthesis.

While conditions in the intestine make for extensive splitting, factors such as presence of alkali, emulsifying agents, wetting agents (bile, monoglycerides), and intestinal movements make for fine emulsification and passage across the absorbing membranes as particulate fat. This direct transport also has some support from histologic evidence (R. M. Wotton and R. L. Zwemer, *Anat. Record* **75,** *493* (*1939*)). It should be noted that the same factors (emulsification, etc.) which make for extensive splitting also favor particulate absorption. The Pflüger school's theory of absorption of the fat only after splitting assumed that the form of absorption was as soaps, but as was shown by B. Moore and D. P. Rockwood (*J. Physiol.* **21,** *58* (*1897*)), and later by others, the pH of the intestinal contents was so much on the acid side that not more than a trace of soap could exist. It was also pointed out that a visible emulsion did not exist for any considerable distance down the tract. The whole question of the solubilizing of the fat was at that time in a very hazy state. The function of the bile and the bile salts was assumed to be mainly that of an emulsifying agent, and it was not until later that F. Verzár and A. Kúthy (*Biochem. Z.* **210,** *265* (*1929*)) showed that the bile salts could combine with the fatty acids to produce water-soluble and diffusible complexes which offered a new mode of absorption of fatty acids.

In contrast to the small amount of work being done on the lipids in America in the first decade of the century was the great interest shown in them on the Continent and especially in Germany. Practically every phase of fat chemistry and biochemistry was being explored by outstanding men. Some of the work is not acceptable at the present time, but a large amount is still good. The synthesis of the triglyceride fats, both simple and mixed, was accomplished by A. Grün and A. von Skopnik (*Ber. d. deutsch. chem. Gesellsch.* **42,** *3750* (*1909*)). They even tackled the synthesis of lecithin with some success. Grün was also interested in the peculiarities of melting point and viscosity of the fats which were later explained on the basis of polymorphism, the fact that the fatty acids and fats can crystallize in more than one form. Each form is characterized by its own melting and solidifying points.

The lipids of brain had been pretty well studied years before by Thudichum and in the present century by O. Rosenheim and M. C. Tebb (*Biochem. Z.* **25,** *151* (*1909*)).

V. H. Mottram (*J. Physiol.* **38,** *281 (1909)*) and later P. Hartley (*Ibid.* **38,** *353 (1909)*) laid the foundation for the understanding of the function of the liver in fat metabolism. Hartley found arachidonic acid as an essential, perhaps *the* essential fatty acid, in the liver. Its exact structure was much later elucidated by D. E. Dolby, L. C. A. Nunn, and I. Smedley-MacLean (*Biochem. J.* **34,** *1422 (1940)*) in England and D. T. Mowry, W. R. Brode, and J. B. Brown (*J. Biol. Chem.* **142,** *671, 679 (1942)*) in this country. H. S. Raper (*Ibid.* **14,** *117 (1913)*) used coconut oil with its high content of volatile fatty acids to throw light on the function of the liver in fat metabolism. C. Dorée and J. A. Gardner (*Proc. Roy. Soc. (London) B* **81,** *109, 230, 505 (1909)*) had begun Gardner's series of studies on the presence and behavior of cholesterol in the animal body. P. G. Unna and L. Golodetz (*Biochem. Z.* **20,** *469 (1909)*) were determining cholesterol and cholesterol esters in skin fats and S. Fränkel and L. Dimitz (*Wien. klin. Wchnschr.* **22,** *1777 (1909)*) worked on the phospholipids of tissues, kidney, pancreas and brain. The physicochemical aspects of the lipids were being investigated by O. Porges and E. Neubauer (*Biochem. Z.* **7,** *152 (1907–1908)*), and an attempt was made by W. Glikin (*Ibid.* **22,** *461 (1909)*) to define the biologic significance of lecithin. M. Kumagawa and K. Suto (*Ibid.* **8,** *212 (1908)*) had worked out a method for determination of lipids in tissues and blood and had done many analyses. Their method is still used. Various methods for cholesterol determinations in blood and tissues were available, most of which have been superseded by later methods.

Windaus had started his series of investigations on cholesterol. In the second decade Levene and associates (P. A. Levene and C. J. West, *J. Biol. Chem.* **24,** *41 (1916)*), especially West, were busy with the lipids of brain, sphingomyelin and its components, sphingosine, the fatty acids cerebronic and lignoceric, and cephalin and its components.

W. R. Bloor
Professor Emeritus of Biochemistry
University of Rochester
School of Medicine and Dentistry
Rochester, N. Y.

EFFECT OF SURGERY ON PLASMA LEVELS OF ESSENTIAL AMINO ACIDS

The possibility that a disturbance in the metabolism of one or more of the essential amino acids is one of the important factors in the pathogenesis of the increased urinary excretion of nitrogen following injury to previously well nourished individuals was suggested a number of years ago (*Nutrition Reviews* **3,** *223 (1945)*). Methionine has been most often considered in this regard, but there are no conclusive data reported regarding methionine or any other amino acid which substantiate this viewpoint.

T. C. Everson and M. J. Fritschel (*Surgery* **31,** *226 (1952)*) have recently studied in 16 patients (specific diagnoses not tabulated) the effect of major surgical operations (in magnitude, cholecystectomy to gastrectomy) on the plasma levels of ten individual amino acids. Fourteen of the patients were stated to be in good nutritional status at the time of operation. Two presumably were not, but the authors do not specifically characterize the nutritional status of these 2 patients.

Plasma levels (free) of arginine, histidine and the eight amino acids considered essential for man were determined microbiologically. Data were obtained preoperatively, immediately postoperatively, and on the morning of the first postoperative day. In half the patients, additional samples were drawn on the third and seventh postopera-

tive mornings. Average figures only are tabulated. Neither total alpha-amino nitrogen in the plasma nor plasma volumes were measured.

The mean plasma concentration of the combined ten amino acids measured was 172 micrograms per cubic centimeter of plasma. This corresponds closely to the level of 181 micrograms per cubic centimeter of plasma reported in healthy medical students by S. W. Hier (Thesis: "Studies on the Free Amino Acids of Blood and Sweat," *University of Illinois (1947)*). Immediately after operation, the mean plasma level of the total ten amino acids was 138 micrograms per cubic centimeter of plasma, a decrease of 34 micrograms, or 20 per cent, from the preoperative control level. This difference is stated to be statistically significant. By the morning of the first postoperative day, however, the level had risen to 161 micrograms per cubic centimeter of plasma, a value not significantly different from the preoperative figure. Similar figures were found on the third and seventh postoperative days, 165 and 161 micrograms per cubic centimeter of plasma respectively.

The preoperative plasma concentrations of the ten amino acids measured were as follows, in micrograms per cubic centimeter of plasma: valine, 29.2; lysine, 27.6; arginine, 21.8; leucine, 20.2; threonine, 18.5; isoleucine, 14.6; phenylalanine, 14.1; histidine, 11.5; tryptophan, 9.1; and methionine, 5.1. Statistically significant decreases in plasma concentration immediately after operation were observed for all but leucine and histidine. The significant average decreases following surgery were as follows: tryptophan, 3.6 micrograms per cubic centimeter, or 40 per cent below the preoperative control value; threonine, 5.8 micrograms, or 30 per cent; arginine, 6.1 micrograms, or 28 per cent; phenylalanine, 3.7 micrograms, or 26 per cent; methionine, 1.3 micrograms, or 26 per cent; lysine, 4.2 micrograms, or 15 per cent; valine, 3.4 micrograms, or 12 per cent; isoleucine, 3.2 micrograms, or 9 per cent. By the morning of the first postoperative day only the values for threonine, arginine, and lysine were still significantly lower than the preoperative levels. The plasma lysine concentration rose to the preoperative level by the third postoperative day, while the values for arginine and threonine rose to normal somewhere between the third and seventh postoperative days.

The transitory fall in the plasma level of the total of the ten "essential" amino acids observed by Everson and Fritschel after major surgical operation is in contrast to the more prolonged decrease in the plasma concentration of total alpha-amino nitrogen following operation reported by E. B. Man and co-workers (Man, P. G. Bettcher, C. M. Cameron, and J. P. Peters, *J. Clin. Invest.* **25,** *701 (1946)*; Man, Bettcher, Cameron, and W. T. Brown, *Yale J. Biol. Med.* **20,** *167, 175 (1947)*; *Nutrition Reviews* **5,** *329 (1947)*; **10,** *99 (1952)*). These workers observed less change postoperatively in malnourished patients with low levels preoperatively than in those patients with normal preoperative levels.

Man and her co-workers felt that the degree and duration of the decline in plasma total alpha-amino nitrogen concentration is related to the severity of the operative procedure. Everson and Fritschel observed no obvious relationship among the type and duration of anesthesia, or the type and duration of operation, and the plasma concentration of the ten individual amino acids measured by them. They point out, however, that the number of patients studied by them is small for comparisons of this nature. To determine whether anesthesia per se might influence the plasma amino acids, 10 dogs were subjected to two hours of ether anesthesia. A lowering of the plasma level of the individual essential amino acids equal to or greater than the depression of plasma levels in the patients produced by anesthesia plus

operation was observed. No data on operated animals are presented.

Everson and Fritschel feel that the decrease in plasma levels of the various individual amino acids observed in the patients immediately after operation probably cannot be attributed to plasma dilution since there was little difference in mean hematocrit or mean serum albumin concentration preoperatively and postoperatively. They recognize, however, the uncertainty of estimating plasma volume from these measurements. No details of fluid or blood administration during or after operation are given. A rise in plasma alpha-amino nitrogen in severe shock and following severe trauma has been described in various species of animals and in human beings (*Nutrition Reviews* **1,** *364* (*1943*); **10,** *99* (*1952*)). Presumably none of the patients in the study of Everson and Fritschel was in shock.

Studies of the urinary excretion of the individual amino acids by 5 of the patients during operation failed to demonstrate an increased excretion sufficient to account for the lowering of the plasma levels of the amino acids.

Considering some of the hormonal factors which might lead to the decrease in plasma levels of the amino acids following surgery or anesthesia, Everson and Fritschel suggest increased secretion of adrenalin as a likely possibility. They point out that C. A. Crismon, R. V. Hanvey, and J. M. Luck (*Am. J. Physiol.* **130,** *171* (*1940*)) have shown that injections of adrenalin will produce lowering of plasma amino nitrogen in fasting dogs. Increased secretion of adrenalin during anesthesia and operation is well established. Although injection of insulin will lower the concentration of alpha-amino nitrogen in the plasma, the amount of insulin required is enough to produce signs and symptoms of hypoglycemia (M. M. Harris and R. S. Harris, *Proc. Soc. Exp. Biol. Med.* **64,** *471* (*1947*); W. D. Lotspeich, *J. Biol. Chem.* **179,** *175* (*1949*)). There is no evidence to suggest this degree of insulin secretion in the immediate postoperative period.

Some of the other endocrine factors which may influence the plasma alpha-amino nitrogen concentrations were discussed recently (*Nutrition Reviews* **10,** *99* (*1952*)). It was noted that C. H. Li, I. Geschwind, and H. M. Evans (*J. Biol. Chem.* **177,** *91* (*1949*)) have shown that the administration of anterior pituitary growth hormone causes a significant decrease in the blood amino acid content of normal rats. This effect occurs within six hours and disappears after twelve hours. This has been interpreted by Li *et al.* as due to the protein anabolic properties of the growth hormone. An observed increase in plasma alpha-amino nitrogen following the administration of adrenocorticotropic hormone has been interpreted by Li and associates as a reflection of its growth-inhibiting properties.

The data obtained by Everson and Fritschel suggest that with the possible exception of arginine and threonine there are no sustained decreases in plasma concentration of the "essential" amino acids during the first week following major surgical operations in previously well nourished patients. Ordinarily, the increase in urinary nitrogen excretion characteristic of the metabolic reaction to operation has reached its peak by this time. This suggests to Everson and Fritschel that there appears to be no direct association between the transient postoperative drop in plasma concentration of the "essential" amino acids (except possibly threonine and arginine) and the postoperative "catabolic" period. It should be mentioned again that a more pronounced decrease in plasma concentration of amino acids was observed by Man *et al.* postoperatively when total alpha-amino nitrogen was measured. The plasma amino acids are presumably in active metabolic exchange with tissue amino acids and tissue and plasma proteins. Studies directed toward correlating these various factors and nitrogen excretion would be helpful.

THE INTESTINAL FLORA OF INFANTS

During the last decade there has been increasing interest in the role played by the intestinal flora in mammalian and, more recently, in human nutrition. Intestinal synthesis of vitamin K is of great importance in maintaining nutriture with regard to this vitamin (*Nutrition Reviews* **3,** *48 (1945)*). The excretion of large amounts of the B-complex vitamins in the feces of experimental animals and man, sometimes quantitatively exceeding the intake (*Ibid.* **4,** *310 (1946)*), would suggest that these supplies might also be available to the host organism.

The role of the intestinal microorganisms in the nutrition of young infants and the design of feeding regimens in relation to the bacterial content of the feces have received little attention from pediatricians since the differences in the feces between nursing and artificially fed infants were originally described at the beginning of the present century. The bacterial flora, hydrogen ion concentration, and sugar content at different levels in the intestinal tract of normal infants fed various cow's milk formulas or their mother's milk have been studied by G. J. Barbero *et al.* (*J. Pediat.* **40,** *152 (1952)*). Seven infants fed human milk and 8 infants fed evaporated milk formulas (containing maltose-dextrin mixtures as carbohydrate supplement) were intubated with a No. 12 infant Miller-Abbott tube which was guided into the duodenum and allowed to pass down the intestinal tract to the rectum. Samples were aspirated from the stomach, duodenum, jejunum, ileum, cecum, transverse portion and splenic flexure of the colon. On each specimen the pH, differential and total bacterial counts were determined. Cultures for the presence of *Lactobacillus bifidus* and determinations of the total reducing sugar were made on several individuals in each group.

The intestinal content of both breast milk- and formula-fed infants was clear, yellow-green fluid until the colon was reached, at which point the fluid became thick and viscous. A fecal odor was noted in material aspirated from the colon of only those infants fed cow's milk mixtures. The pH was essentially the same in both groups in the small intestine, acid reaction in the stomach, approaching neutrality in the duodenum, with a slow rise to a peak between 6.5 and 8.0 in the cecum. In the large bowel a difference between the two groups was noted. There was a steady pH fall in the breast milk-fed group with the production of more acid feces than those formed by infants fed evaporated cow's milk. There was a greater variation throughout in the artificially fed group.

The duodenum was relatively sterile and the bacterial population increased in both groups in the rest of the small intestine. Differential counts showed that the flora consisted largely of enterococci until the terminal ileum was reached. The artificially fed infants had a higher percentage of gram-positive bacilli in the cecum than in the feces which contained appreciable numbers of gram-negative rods, while the infants fed breast milk had a gradually predominating gram-positive flora in the large bowel and over 90 per cent of these organisms in the feces. Cultural studies of material from the large bowel revealed bifid gram-positive bacilli (presumably *L. bifidus*) in the majority of the samples from the ascending and transverse colons of both groups. However, bacteria with this morphology were cultured from the feces of only 2 of 5 infants fed cow's milk while they were present in the feces of 5 of 6 breast milk-fed infants. Reducing sugar was present in various amounts at all levels of the bowel in the 5 infants studied.

The effects of diet on the pH and percentage of gram-positive bacilli in the feces were observed in a much larger group of

infants. Twenty-two infants fed human milk had acid feces averaging pH 5.5 and containing 97.4 per cent gram-positive rods. Evaporated milk feeding of 130 infants resulted in a fecal pH of 6.7 and 53.4 per cent gram-positive organisms. Three proprietary "simulated breast milk" preparations were also studied. The feces from infants fed these preparations had a pH similar to that of feces from infants fed evaporated cow's milk. A modification of one product in which the shorter chain unsaturated fatty acids of the series C^{10} to C^{14} were reduced to the lowest possible levels was also studied. The depressing effect of these acids on the growth of strains of *L. bifidus* has been reported by some of these same workers (R. M. Tomarelli, R. F. Norris, C. S. Rose, and P. György, *J. Biol. Chem.* **187,** *197 (1950)*). This modification has resulted in feces having a pH closely approaching that found with human milk, but the percentage of gram-positive bacilli was not so great as that found in the latter group.

The predominance of *L. bifidus* flora and the greater acidity of the feces of breast-fed infants as contrasted with the variable flora and higher pH of the feces of infants fed cow's milk are confirmatory of previous studies. However, it is pointed out that the feces are the end result of a long series of microbiologic reactions. The effects of intubation and the admitted inaccuracies of some of the methods available make the preliminary nature of these studies obvious.

The biochemical and nutritional factors governing the proliferation of *L. bifidus* and other lactobacilli deserve further study since the morphologic characteristics of this organism are necessarily the major criteria used for its identification at the present time. Much remains to be learned concerning both the factors in the diet which play a determining role in the development of the characteristic intestinal flora and the relative importance of this flora in the nutrition of the host. These studies reflect increasing interest in these factors in infant nutrition, and it is hoped that much useful information will be obtained concerning both normal and pathologic conditions by use of similar technics.

RESTRICTION OF FOOD INTAKE AND HEALTH

In the history of medical thought and experience two opposite trends may be noted as far as the relationship of dietary restriction and health is concerned. On the one hand, moderation in food or an occasional fast has been considered to have beneficial influence on the maintenance of health and a curative power at least in the case of some diseases. On the other hand, starvation is known to be associated with increased incidence and virulence of some infectious diseases, such as dysentery, typhoid, cholera, and especially tuberculosis.

The clarification of the basic issues and the separation of spurious and real correlations is not a simple task. The ideas about the effectiveness of fasting are frequently tied up with systems of mythologies. Experimental investigations in this area, for several reasons, are difficult. Some authors are inclined to believe that at least part of the effectiveness of the rice diet in the treatment of hypertension is due to the concomitant caloric deficit.

Prolonged fast as treatment for cardiac decompensation was explored by G. Mauro and M. T. Borello (*Minerva Medica* **41,** *Vol. II, 249 (1950)*). They studied 21 patients with cardiac muscular and valvular lesions. Fasting was imposed for one week (six to eight days), with no limitations on the water intake. The patients remained in

complete bed rest. Improvement in the general status was noted after the first three or four days of treatment, with diminution of palpitation, cough, precordial pain, and dyspnea. Improvement in subjective symptoms was paralleled by objective criteria: such as reduction of the heart rate; increased diuresis in patients with predominance of right heart decompensation, the urine output being related to the amount of edema present at the start of the treatment, with a maximum weight loss of 13 kg.; increased urinary chloride excretion; reduction of arterial, especially diastolic, blood pressure in hypertensive patients, of venous blood pressure, of the circulation time, and of nonprotein nitrogen in the blood; improvement in the electrocardiogram, and on x-ray examination (*e.g.*, reduction of the heart diameters). It is the authors' opinion that the benefits derived from the fast therapy cannot be explained in terms of the diuresis and the resulting lessening load on the circulation. No concrete information is provided on the duration of the improvement obtained, but the authors have noted that by a sparing use of cardiotonic drugs (digitalis) the state of compensation may be perpetuated for a considerable length of time following the fast. The treatment is undoubtedly a drastic one and in some cases it had to be terminated as early as the third day. The authors stressed the need for constant medical supervision during the period of the fast therapy.

The last war and the undernutrition associated with it resulted in largely unpremeditated mass experiments on the effect of prolonged undernutrition on health. Several articles in *Nutrition Reviews* have been concerned with this topic, sometimes only incidentally (**6,** *293 (1948)*; **8,** *304 (1950)*). The literature on undernutrition and infectious diseases, with a special chapter devoted to tuberculosis, to diabetes, and to cancer was summarized and evaluated in the second volume of "The Biology of Human Starvation" by A. Keys *et al.* (*p. 1002, Univ. of Minnesota Press, Minneapolis (1950)*).

A statistical study, not incorporated in the above monograph, on the effect of wartime dietary restriction on health in the Scandinavian countries was reported by H. Malmros (*Acta med. Scandinav., Suppl.* **246,** *137 (1950)*). Limited data are presented on diabetic morbidity and mortality in Sweden. During the lean war years, especially in 1942 and 1943, the death rate per 100,000 population reached the lowest values observed during the decade 1935 to 1945, although a slight but fairly regular decline had been observed since 1935. The morbidity data are more complete and show a more striking decline from 1939, with the minimum in 1942 and 1943, followed by a rise in the subsequent years. The total number of cases of diabetes mellitus treated in 22 medical clinics was about 2250 in 1939, 1880 in 1942, 2480 in 1944, and 2640 in 1946. The subsequent rise in diabetic morbidity during the years when food again became plentiful is taken as evidence that the observed drop was due to reduced food consumption and not to other factors influencing the hospitalization of patients. No data on the actual shifts in the caloric consumption are given, but the Swedish mortality and morbidity figures fit in well with the universally observed favorable effects of a moderate degree of caloric restriction on the incidence and course of the disease.

The effect of undernutrition on tuberculosis mortality is less uniform, even though a markedly increased rate is most common. The increase was dramatic in some parts of Europe, and tuberculosis morbidity and mortality reached fantastic proportions in the infamous German concentration camps. In the study reported by C. Debray *et al.* (*Semaine d. hôp. Paris* **22,** *863 (1946)*), out of 771 men, largely political prisoners, 52 per cent had tuberculosis. Estimates have been made that some 40 per cent of deaths in the Buchenwald concentration camp were

due to tuberculosis. In Berlin, according to G. Berg (*Svenska Läkartidningen* **46,** *2201 (1949)*), the mortality in 1945 was 264 per 100,000 inhabitants as compared with the prewar rate of 82 in 1938; in 1947 it dropped to 224. Malmros noted that this rise in tuberculosis mortality cannot be ascribed solely to nutritional factors. Overcrowding, deficient hygiene, and displacement of large portions of the population have increased the risk of infection. But even this does not tell the whole story. In Greece, which went through severe famine in 1941 to 1943, there was only a minimal rise in the incidence of tuberculosis (T. Doxiadès, *Rev. méd. de la suisse rom.* **70,** *224 (1950)*; *Nutrition Reviews* **10,** *60 (1952)*). In Norway, where food supplies were very scanty during the war years although the country had not experienced a real famine, the tuberculosis mortality figures steadily declined from 1941 through 1945 with the rates of 80, 73, 69, 74 and 65 per 100,000 for the five years. The average value for the preceding five years was 91. These data indicate that a *moderate* reduction of the calorie supply does not lead necessarily to increased tuberculosis mortality.

The core of Malmros' paper was concerned with the relationship of diet to mortality from arteriosclerosis including sclerosis of the coronary arteries. The unreliability of any single listed cause or a combination of such causes of death as indices of the status or course of vascular disease is recognized and discussed. To avoid the uncertainties of comparison of mortality rates obtained in different countries and thus according to different standards, Malmros draws his conclusions largely from a consideration of successive annual changes of the cholesterol content of the diet and mortality rates within countries. Conditions precipitated by the war led to significant variation in the total dietary fat and cholesterol in Sweden, Finland, Norway and Denmark.

The author believes these data indicate that a reduction of cholesterol-rich foods in the diet is *immediately* associated with a decline of arteriosclerosis mortality. The performance of Sweden, Finland and Norway is cited to support this contention. The author's conclusion, however, is not based upon clear or convincing evidence because many other dietary changes were introduced during the periods of supposed changes in cholesterol intake.

In Sweden, an apparent decline of arteriosclerotic mortality during the lean years of 1942–1943 is related by the author to a decrease in per capita egg consumption. Similar data obtained in Finland and Norway lead the author to the same conclusions. By contrast, in Denmark, where the war conditions did not diminish the average cholesterol intake, a steady increase in arteriosclerotic deaths is cited. Similar evidence is cited from United States statistics.

Malmros' ideas do not offer an explanation of the higher arteriosclerosis mortality in Sweden over that of the United States, despite a lower per capita consumption of both total fat and cholesterol in Sweden.

Malmros comments on the notable lack of a latency period between changes in the average diet of the various Scandinavian countries studied and the observed mortality rate alterations during the wartime food restriction. This important point has recently been discussed by D. Barr and others (*Am. J. Med.* **12,** *357 (1952)*). While there does seem to be room for optimism concerning control of the rate of development of arteriosclerosis, there can be little doubt that the rate of change of the disease is slow, and measured in years rather than weeks or months. Barr has made the interesting conjecture that a thromboembolic mechanism may be of particular significance in precipitating the clinical catastrophes of arteriosclerosis. In this fashion the most immediate influences of

diet may be upon the clotting mechanism which in return reflects itself in the clinical manifestation of the much slower moving entity arteriosclerosis. An analogous situation has been observed in the influence of influenza pandemics upon tuberculosis mortality, the latter rising precipitously because of the predilections of tuberculosis subjects to fatal influenza complications.

The apparent correlation between diet and disease described by Malmros, while perhaps real, does not constitute grounds for recommendation of sweeping dietary changes in the countries studied.

NUTRIENT ABSORPTION FOLLOWING INTESTINAL RESECTION

With continuing improvement in surgical technic and postoperative care successful removal of massive amounts of small intestine for various disease states is now becoming more common. The increased survival rate in this type of surgery thus presents the clinician with the heretofore unusual problem of providing adequate nutrition for patients with limited absorptive capacities. How limited these capacities are for various nutrients and how much adaptation occurs in this type of situation have been given rather scant attention. Several studies, however, are available which appear worthy of summary.

In a comparison of the digestion and absorption of diets of a "natural" type and of a "synthetic" type, T. L. Althausen, K. Uyeyama, and R. G. Simpson (*Gastroenterology* **12,** *795 (1949)*), undertook balance studies of a woman whose remaining intestine after surgery was composed of duodenum, 15 cm. of jejunum and the large intestine. Ten months after surgery, balance studies over four-day periods were carried out while she was receiving: (1) a diet composed of ordinary foods, and (2) a formula composed of protein hydrolysate, cream, glucose, milk and cocoa. Two days were allowed for acclimatization to the diets before the four-day collections were begun. Although the diets were similar in their nutrient composition, varying amounts of each were refused, and more protein and calories were ingested on the synthetic diet than on the natural diet.

Nitrogen intake of the first diet was 41.1 g. Urine and fecal nitrogen during this period were 24.7 and 15.5 g. respectively. During the second diet the intake was 54 g. of nitrogen, while urine and fecal nitrogen were 26.7 and 25.5 g. respectively. Fat intake and fecal fat were 194 g. and 149 g. respectively during the first study and 183 g. and 131 g. during the synthetic diet, while 5382 and 6094 calories were ingested during the respective diet periods. It thus becomes apparent that there was little difference in the metabolism of the nutrients provided by the two regimens except for slightly better percentage absorption of protein from the natural foods.

Despite the small differences observed, this single study is of some interest in view of the fact that the majority of the protein ingested on the second diet was in the form of amino acids, theoretically more easily assimilable than whole protein. It is also noteworthy that the authors report that the patient refused to continue taking the synthetic formula despite the better nutrient intake accomplished by its use.

In separate studies of absorption conducted on this patient, methionine, vitamin A, galactose and oral glucose tolerances were all lower than normal. Intravenous glucose tolerance was normal, however, serving to confirm the conclusion that absorption was the primary defect in this patient with little residual intestine.

In a subsequent investigation of two similarly operated patients T. L. Althausen,

R. K. Doig, K. Uyeyama, and S. Weiden (*Gastroenterology* **16,** *126 (1950)*) attempted to assess the recovery by the intestinal tract of certain functions. Serial absorption tests were carried out on a woman with 61 cm. of jejunum plus the colon remaining, and on a man with approximately 46 to 61 cm. of jejunum, a like amount of terminal ileum, and the colon remaining. Both had had diarrhea immediately after operation which had markedly decreased in volume and fluid content by four to six weeks following surgery.

In the first patient, oral glucose tolerance was low one week after operation, but was normal at six weeks. The second patient, however, had a delayed maximum rise of blood sugar and prolonged elevation of the curves at both one month and six months after surgery despite normal intravenous glucose tolerance on both occasions. Oral galactose tolerance in the first patient was successively low, low normal, and elevated above normal at six weeks, six months and twenty months respectively. This test was not carried out on the second subject.

Oral methionine tolerance tests at six days, six weeks and five and one-half months were 17 per cent (of "average normal"), 88 per cent, and 87 per cent respectively in the first patient. Glycine tolerance at twenty months in this patient was 74 per cent of average normal. This test in the second subject was 35 per cent at one month and 107 per cent at six months.

Fat absorption was tested by vitamin A tolerance and "butter tolerance" in the first patient and by the latter test alone in the second patient. Vitamin A tolerance revealed no improvement, with figures of 36, 35 and 22 per cent of average normal being recorded at six weeks, five months and twenty months respectively in the single patient tested. Simultaneous use of the detergent "Tween 80" with vitamin A resulted in a rise in this test to 76 per cent at five months, but after prolonged use as a therapeutic agent Tween 80 did not improve the results at twenty months as compared with the figure for vitamin A administered alone. The butter test, which consists of measuring the serum turbidity for six hours following the oral ingestion of 60 g. of butter, was 74 per cent of average normal at twenty months. In the second patient marked improvement, from 9 per cent at one month to 114 per cent at six months, was found by this procedure. These results are interpreted by the authors as demonstrating a difference in the absorption of different types of fat and improvement in fat absorption. It appears, however, that the data are too limited to permit any but the most reserved statements concerning changes in lipid absorption in these patients.

Despite the paucity of data available from these studies it appears that defective absorption is most marked in regard to fat in patients with small amounts of functioning intestine. Carbohydrate and protein absorption appear to be diminished to a lesser degree. These results have been confirmed in a study by L. G. Berman, H. Ulevitch, H. H. Haft, and S. Lemish (*Ann. Surg.* **132,** *64 (1950)*) of a woman with but 45 cm. of jejunum remaining after surgery. Balance studies on their patient six months after surgery revealed fat absorption to be most markedly defective while nitrogen was affected to a lesser extent and glucose absorption as measured by oral tolerance test was normal. It is of interest that this patient was able to hydrolyze fat well, but that free fatty acids were in excess of the normal as compared to soaps and neutral fats in the feces.

These investigations not only confirm previous studies of this type of clinical problem, but also suggest that "synthetic" diets have no advantage over natural types of foods and that a certain degree of accommodation or recovery occurs with the passage of time in patients with decreased intestinal surface area. Dietary therapeutic

efforts should be directed at maintaining a high carbohydrate, high protein, and low fat intake to avoid the damaging effects of steatorrhea. In view of the reported results with the use of ACTH and cortisone in other types of steatorrheic patients (*Nutrition Reviews* **10,** *169 (1952)*), a trial of these agents might be of interest in the surgical problems reviewed. Most necessary however, is expansion of this type of study to include data on the absorption of other nutritional factors.

GASTROINTESTINAL X-RAY STUDIES IN THIAMINE DEFICIENCY

The alterations in blood levels of thiamine in elderly individuals have been the subject of intensive investigation by J. E. Kirk and M. Chieffi (*see Nutrition Reviews* **9,** *175 (1951)*). A significantly greater incidence of hypothiaminemia (low blood thiamine level) has been found in older persons by these workers and this finding has been associated with certain symptoms and physical signs generally agreed to be characteristic of B-vitamin depletion states. In a recent extension of these studies, R. W. Maxwell, Chieffii, and Kirk (*Gastroenterology* **20,** *309 (1952)*) have made use of gastrointestinal radiography in evaluating nutritional status. The deficiency signs observed in the small intestine have been summarized by R. Golden (*Radiology* **36,** *262 (1941)*), and have been widely accepted, although they are nonspecific in regard to the type of nutrient involved.

In this study, 20 patients 51 to 90 years of age whose blood thiamine values ranged from 0.0 to 2.4 micrograms per cent were studied by roentgenography after barium administration. The results were compared with similar roentgenograms performed on 20 middle-aged and elderly individuals whose blood levels were 3.5 micrograms per cent or higher. Of the hypothiaminemic patients, 9 had edema, 4 had edema and tenderness of the calves, 1 had edema and vibration sense impairment, 1 had impaired vibration sense and tenderness of the calves, and 2 had impaired vibration sense only. No gastrointestinal symptoms were present and all had had low thiamine levels for five months while receiving a diet containing by analysis 1.5 mg. of thiamine daily.

Pretreatment x-rays showed only minor and insignificant changes in the mechanics and appearance of the small intestines of the hypovitaminemic patients and did not differ markedly from those of the control series. After the initial study, all patients were given 5 mg. of thiamine hydrochloride daily by mouth for three months. At the end of this time all had blood thiamine levels of 3.5 mg. per cent or greater and in all except one patient each of the above-mentioned deficiency signs had disappeared. Repetition of the x-ray examinations was carried out at this time, but no significant alterations were observed as a result of therapy.

The authors point out that these results do not exclude the possibility that more severe deficiency may produce radiologic evidence of gastrointestinal abnormality. It should also be emphasized, however, that absolute levels of thiamine do not appear, on the basis of these studies, to indicate the severity of the disease in a given individual. Thus, although individual values are not given, the range of thiamine levels indicates that at least one member of the depleted group had no vitamin detectable in the blood. The absence of gastrointestinal changes in patients with this degree of depletion therefore suggests that thiamine

levels are still a rather crude method of measuring the severity of a patient's hypovitaminosis. Further information on this subject might be gained in studies of patients with positive x-ray changes who are subjected to therapy and repeatedly examined by both x-ray and blood thiamine levels. An additional point which can be gleaned from this investigation is the apparent lack of correlation of the physical signs with the gastrointestinal changes. If pure thiamine deficiency can produce alteration in the small bowel as seen on x-ray it is obvious that the bowel is markedly affected only after pronounced physical changes are present.

FEEDING PROBLEMS DURING FLIGHT

The rapid increase in flying has accentuated the problems of feeding both personnel and passengers on aircraft. Although an important consideration for commercial aircraft, complexity increases in military flying. Some of the problems and their solutions have been reviewed (*Nutrition Reviews* **9,** *254* (*1951*)). Further discussions of this problem have been presented by H. C. Dyme (*J. Aviation Med.* **22,** *146* (*1951*)) and W. E. Bills (*Ibid.* **22,** *418* (*1951*)). Repeated emphasis is placed upon non-nutritional problems, such as military strategy, aircraft facilities, sanitation, and the growing complexity of military air missions. Nutritional considerations for short or even moderately long flights are considered less important than supplying "quick energy" and contributing psychologic benefit. The "Food Packet, Individual, Combat, In-Flight (IF-2)" briefly described in the previous review (*Nutrition Reviews, loc. cit.*) has been found highly satisfactory, appetizing, and simple to transport.

The feeding of the fighter pilot during flight exaggerates the difficulties of in-flight feeding. Since the trend is to increase the range of fighter aircraft, such as jet fighters equipped for air refueling, feeding becomes an increasingly important consideration. Here again the emphasis is upon carbohydrates, which are believed to produce a slight increase in altitude tolerance. It has been pointed out that digestive processes are usually inhibited during combat so that foods supplied must be simple as well as appetizing and they must quench thirst.

When one considers that the cockpit in these aircraft is designed to house the pilot and instruments in a small space, that the pilot will be using oxygen continuously at high altitudes and may be dressed in "an immersion suit" and "winter flying clothing," and finally that high speeds produce a marked increase in gravitational forces at the end of dives, the difficulties in providing food are apparent. So far, fruit juices, chocolate drinks, and soups have been the most satisfactory foods tried. However, to keep soups or other drinks hot has been another problem. So far, no truly satisfactory method of heating food or of keeping it hot, or of finding a suitable spot in the cockpit for storage of hot food or apparatus for heating food has been found. A great deal of work is being done on this matter at present.

More work is planned, not only upon the matter of placement of food in a cockpit and maintaining its warmth, but also upon the effect of flight and its concomitant abnormalities of environment on the physiologic utilization of food, especially on absorption and intermediary metabolism.

THE SOURCE OF LYSINE, TYROSINE AND PHOSPHORUS FOR CASEIN SYNTHESIS

A recent study (*Nutrition Reviews* **10,** *189 (1952)*) demonstrating that carbon dioxide fixation plays a role in the synthesis of casein by the cow has been described (M. Kleiber, A. H. Smith, and A. L. Black, *J. Biol. Chem.* **195,** *707 (1952)*). Another recent publication (J. M. Barry, *Ibid.* **195,** *795 (1952)*) now throws much needed light on the source of two amino acids, lysine and tyrosine, and of phosphorus for casein synthesis by the mammary gland itself.

One older report (A. H. W. Aten and G. Hevesy, *Nature* **142,** *111 (1938)*) had suggested that the mammary gland takes inorganic phosphorus from the blood stream to provide casein phosphorus, but did not exclude the possibility that inorganic phosphorus might be rapidly converted to some other compound, in another part of the body, before being absorbed by the mammary gland. There was no evidence for the source of the lysine and tyrosine of casein. In particular, it was not known whether the mammary gland uses the free lysine and tyrosine of the blood to provide all the lysine and tyrosine of casein, or whether plasma proteins were broken down in this tissue to provide these amino acids.

Barry carried out two types of experiments. The first was done on a goat which gave about 1400 ml. of milk per day at the height of lactation and was in good health. The goat was milked out, pituitrin—which causes intense contraction of the mammary gland—being injected to insure completeness of the process. A radioactive solution, containing sodium orthophosphate labeled with P^{32}, and lysine and tyrosine labeled with C^{14} in the carboxyl group, was injected into the left jugular vein. Milk was collected at one and one-half, three, four and one-half, seven, nine, and twelve hours after injection, only milk from the right teat being used for analysis. Blood samples were taken from the right jugular vein.

Casein was separated from the centrifuged milk by acetic acid precipitation, and hydrolyzed with hydrochloric acid. The resultant amino acid mixture, properly neutralized, was submitted to the action first of tyrosine decarboxylase, next to that of lysine decarboxylase. The carbon dioxide was collected during each process and total amounts and specific activity were determined. Similar procedures were conducted on plasma proteins. The specific activity of blood lysine and tyrosine was also determined, in the plasma-free filtrate, by use of the specific decarboxylases. The activity of both the inorganic and the organic phosphorus fractions was determined after precipitation of the inorganic phosphorus as the magnesium ammonium salt, extraction of lipid phosphorus with ethanol-ether mixture, and solution of labeled casein in borate buffer.

It was found, by consideration of the curves giving specific activities of plasma and milk phosphorus as functions of time after injection (*in vivo* experiments with the goat), that the specific activity of the inorganic phosphorus in the milk, after it has risen to a maximum, is always equal to that of the plasma inorganic phosphorus about one and one-half to two hours earlier. Similarly, the specific activity of the casein phosphorus is always equal to the activity of the plasma inorganic phosphorus about two to two and one-half hours earlier. During the latter part of the experiment, the specific activity of the inorganic phosphorus of blood stayed constant and was almost identical with that of milk inorganic phosphorus and casein phosphorus. Barry concludes from these observations that all of the casein phosphorus is derived from the inorganic phosphorus of blood, and that the

delay in incorporation is about two to two and one-half hours.

By similar considerations, it was possible to demonstrate that all of the casein lysine and tyrosine is derived from the free lysine and tyrosine of blood, the delay of incorporation being on the order of one and one-half to two hours.

The remaining possibility, that the mammary gland, instead of taking inorganic phosphorus and free lysine or tyrosine and synthesizing the casein from these materials within the calculated delay, receives intermediary substances manufactured in other parts of the body during this two-hour interval, was eliminated by a second type of experiment. In this series, the excised left half of the udder of a normally lactating cow was perfused with citrated blood containing labeled (P^{32}) orthophosphate and labeled (C^{14}-carboxyl) lysine and tryosine. The same analytic procedures were applied. Again it was found that the specific activity of the phosphorus, lysine and tryosine extracted from the secreted casein was such as to prove that they were derived entirely from the inorganic phosphorus and the free corresponding plasma amino acids.

Essential amino acids constitute about 50 per cent of the amino acids of casein. Arteriovenous differences in amino acid nitrogen across the lactating mammary gland can account for at least 50 per cent of the casein nitrogen. Barry extrapolates from his findings the conclusion that this arteriovenous difference in amino acids must reflect for a large part the uptake of essential amino acids for milk protein synthesis. It seems that the next step in this interesting study could be a correlation of the uptake in essential amino acids with the uptake or the synthesis, by the lactating mammary glands, of the nonessential amino acids. The rate of synthetic activity of the mammary gland is such that, by extending the methods used in these experiments, it should be possible to elucidate under better conditions than with any other mammalian tissues important aspects of the mechanism of protein synthesis.

NIACIN AND TRYPTOPHAN IN FELINE NUTRITION

The domestic cat has been used very little in nutrition studies. Consequently very little is known about its nutritional requirements as compared with the requirements of other species.

A recent study by A. C. da Silva, R. Fried, and R. C. de Angelis of Sao Paulo, Brazil (*J. Nutrition* **46,** *399 (1952)*), suggests that the domestic cat is different from many other mammalian species in that it is unable effectively to convert tryptophan into niacin.

Animals used in the study were 8 to 10 weeks of age when purchased and were fed a normal stock diet until they were well adapted to the laboratory. They were then placed on an experimental diet of the following percentage composition: "vitamin-free" casein, 35; gelatin, 1; sucrose, 48; peanut oil, 5; lard, 5; salt mixture, 5; and "Ruffex," 1. This mixture was fed as a paste by wetting with water. Vitamins were supplied in the following amounts (milligrams per week per cat): thiamine, riboflavin, pyridoxine, vitamin K and para-aminobenzoic acid, 3 each; calcium pantothenate, 12; folacin, 1.5; D-biotin, 0.15; inositol, 90; choline, 900; and alpha-tocopherol, 15. In addition, each cat received 6 ml. of cod liver oil per week. Ascorbic acid had been found in earlier trials not to be required.

When this diet was supplemented with 12 mg. of niacin per cat per week, the animals were maintained in good health for periods of at least twelve months. When the

diet was fed without the niacin to young cats, they ceased growing in ten to fifteen days and usually died in four to seven weeks. Adult animals usually lost weight on the unsupplemented diet and also eventually died. In addition to growth failure, the niacin-deficiency symptoms noted were an unthrifty fur and severe diarrhea. Respiratory diseases frequently contributed to early death in the niacin-deficient young animals. Evidence of anemia and skin or mouth lesions was not observed.

Young cats depleted of niacin and losing weight exhibited prompt recoveries when niacinamide was administered. One- to three-milligram single doses given subcutaneously produced a total weight gain of 100 to 200 g. over a four- to eight-day period. The precise quantitative requirement was not established, but the optimum response appeared to result from ten-milligram doses of niacinamide.

Of unusual interest was the observation that the niacin deficiency in cats could not be corrected with tryptophan. The cat was unable to convert tryptophan to niacin or its metabolites, a process known to occur in the species thus far studied, namely, mouse, chick, guinea pig, calf, horse, pig, rabbit and man. Whereas a marked increase of urinary N-methyl-niacinamide was routinely observed by the Brazilian workers when niacinamide was administered, no increase in the excretion of this metabolite was detected when tryptophan was administered either orally or subcutaneously in doses of 40 to 600 mg. The authors, however, pointed out that the metabolic data obtained could not be considered as proof that no conversion of tryptophan to niacin had occurred. The data do support the view that if such conversion occurs it apparently occurs to a much smaller degree than in other mammals.

That cats metabolize tryptophan in a manner different from other mammals was confirmed by the observation of the Brazilian workers that no xanthurenic acid could be isolated from the urine after the feeding of high protein diets deficient in pyridoxine. They postulate that the degradation of tryptophan in cats follows the pathway characteristic of other species up to kynurenine (*Nutrition Reviews* **8,** *85 (1950)*), but differs from this stage onward.

That a diet containing as much as 35 per cent casein could not prevent the development of niacin deficiency in cats suggests strongly that if tryptophan is converted to niacin in the cat, the mechanism is not very efficient. The statement made by the Brazilian workers that the niacin requirement of the rat is satisfied by diets as low as 8 per cent in casein must, however, be questioned. Diets containing as much as 18 per cent casein will stimulate growth in rats when supplemented with niacin (W. D. Salmon, *J. Nutrition* **33,** *155 (1947)*).

The studies with cats are of great interest and again emphasize that the nutritionist must resort to a variety of species in experimental animals in order that the function and metabolic pathways of essential dietary nutrients can be clearly established.

THE ACTION OF THYROID, CHOLINE AND INOSITOL ON TISSUE CHOLESTEROL AND LIPIDS

The thyroid hormone causes a lowering of serum cholesterol in man and certain experimental animals. The effect of thyroid on tissue cholesterol has not been so extensively studied. W. L. Marx, L. Marx, and F. Shimoda (*Proc. Soc. Exp. Biol. Med.* **73,** *599 (1950)*) have examined the effect of thyroid and thiouracil feeding on liver as well as plasma cholesterol concentration of rats fed 1 per cent cholesterol and 0.5 per

cent bile salts in their diet. The changes in plasma cholesterol were the expected fall during thyroid feeding and rise during thiouracil administration. The cholesterol concentrations of the liver were increased by cholesterol feeding and to a great extent were independent of thyroid hormone or thiouracil treatment. The aorta was observed for cholesterol deposits, and in animals fed cholesterol there was a moderate cholesterol deposition. The deposition of cholesterol was not lessened by thyroid feeding.

The lipotropic effect of thyroid hormone and its relation to choline and inositol has been the subject of a recent report by S. B. Weiss, L. Marx, and W. Marx (*Endocrinology* **50,** *192 (1952)*). In these studies immature female rats were fed a synthetic high fat diet for seven weeks. The effect of the addition of the following supplements, either individually or in combination, was observed: (1) 2 per cent cholesterol, (2) 0.4 per cent thyroid, and (3) choline and inositol, either 0.25 per cent or 1.0 per cent.

This diet without the addition of cholesterol caused no change in the plasma cholesterol, but the liver cholesterol concentration rose from 0.21 per cent to 1.12 per cent. The total liver lipid rose from 5.9 per cent to 21.5 per cent. The incorporation of either thyroid or choline and inositol was effective in lowering the hepatic cholesterol and lipid content. Choline plus inositol proved more effective in lowering hepatic lipid than did thyroid.

The addition of 2 per cent cholesterol to the basal diet was followed by an increase in plasma cholesterol from 60 to 107 mg. per cent. The total hepatic lipids increased from 21.5 per cent without cholesterol to 37 per cent with cholesterol in the diet. Liver cholesterol rose from 1.12 per cent to 10.0 per cent. The addition of thyroid powder to the diet was followed by a lowering of the plasma cholesterol and liver cholesterol and total lipid. When choline and inositol were added to the basal diet there was an *increase* in plasma cholesterol and a *decrease* of liver cholesterol and total lipid. When thyroid, choline and inositol were administered concomitantly the greatest lowering of hepatic cholesterol and lipid occurred.

The much greater effect exerted by thyroid hormone on cholesterol concentration in the liver as reported in the more recent publication of this group can probably be explained by the great increase in fat in their experimental diet and a mild deficiency of choline and choline precursors. It is regretted that the authors did not evaluate independently the roles of choline and inositol. In view of the results of C. H. Best, J. H. Ridout, J. M. Patterson, and C. C. Lucas (*Biochem. J.* **48,** *448 (1951)*; *Nutrition Reviews* **9,** *329 (1951)*), a lipotropic effect of inositol on hepatic lipid would not be expected with a high fat diet. It thus seems probable that the lipotropic action observed by Weiss *et al.* could be ascribed to the choline rather than to the inositol supplementation.

The hypercholesterolemia which occurred in rats fed a high cholesterol diet supplemented by choline and inositol is an unexpected finding. The bulk of the existing literature would lead one to expect a lowering or no change in the cholesterol content with these agents. A similar aggravation of cholesterolemia, however, has been reported in chickens fed cholesterol in their diet.

These studies by Weiss and his collaborators have demonstrated a real lipotropic effect of thyroid hormone. Their findings re-emphasize the independence of serum and tissue cholesterol levels and the importance of the experimental conditions in determining the nature of the effect of lipotropic agents. Further study of the interrelation of thyroid hormone and lipotropic agents should prove fruitful.

INFLUENCE OF ROUTE OF ADMINISTRATION AND OF SEX ON SERUM IRON LEVELS

Blood contains, in addition to the iron present in the blood as a constituent of hemoglobin, a small quantity (approximately 100 micrograms per cent) of iron present in the blood as such. This "serum" or "plasma" iron is not dialyzable at physiologic pH. The demonstration by C. G. Holmberg and C. B. Laurell (*Acta physiol. Scandinav.* **10,** *307 (1945)*) that there is in serum a protein component capable of binding iron in a firm complex linkage marked the beginning of extensive research on serum iron. The iron-binding protein was shown, electrophoretically, to be a $beta_1$-globulin, of a molecular weight of approximately 90,000. The globulin was isolated and shown to combine not only with iron but also with copper and with zinc. When iron is added to a solution of the crystallized globulin, a real complex is formed which can be characterized by its absorption spectrum (D. M. Surgenor, B. A. Koechlin, and L. E. Strong, *J. Clin. Invest.* **28,** *73 (1949)*). The plasma content of this iron-binding protein was studied in a number of patients suffering from various types of anemias (*Nutrition Reviews* **7,** *231 (1949)*).

Although the existence and characteristics of this iron-binding globulin had thus been definitely established, it was claimed by some workers that it represented only one of several alternative proteins concerned with iron transport in the serum. For example, it was claimed that iron sometimes migrates electrophoretically with the albumin peak, or with several of the plasma protein peaks. F. Thedering (*Acta haematol.* **3,** *210 (1950)*) was even more specific. According to this author the route of administration of iron conditions the type of protein binding of the metal. Iron given by mouth is predominantly bound to albumin. Injected iron is combined mostly with globulin. When iron is added to serum *in vitro,* it is linked to the serum albumin.

The availability of radioactive iron has allowed G. Wallenius (*Scandinav. J. Clin. Lab. Invest.* **4,** *24 (1952)*) to solve this controversy, in the case of the laboratory rat as regards *in vivo* experiments, and with human serum *in vitro.* Wallenius gave radioactive iron both by mouth and parenterally to his experimental animals. Paper strip electrophoresis (G. Schneider, *Acta chem. Scandinav.* **5,** *1020 (1951)*) then allowed him to separate the various blood proteins in bands which can be stained, extracted for spectrophotometric characterization, or assayed for radioactivity by exposing a roentgen film to the paper strip. He found that, irrespective of the route of administration, the added iron migrated with the beta-globulin fraction. Furthermore, the fact that all the iron moved toward the anode proved that all of it was bound to the protein; free iron would have travelled toward the cathode. The addition of iron to human serum *in vitro* gave rise to similar results.

As the amount of iron he used was small enough not to saturate the binding capacity of the specific iron-carrying globulin, Wallenius points out that his experiments do not necessarily exclude the possibility that, when very large amounts of iron are given, other proteins may also combine with it. Wallenius' report seems, however, to establish definitely the mode of serum iron transport under normal physiologic conditions.

Also of interest in connection with serum iron levels is a recent note (H. Palmer, *Scandinav. J. Clin. Lab. Invest.* **4,** *81 (1952)*) concerned with the influence of sex hormones on the concentration of iron in the plasma. Lower values had previously been found in

women than in men, with menstrual losses considered to be responsible for the difference. Palmer found, however, that the plasma iron level of mature and healthy women who had been hysterectomized for benign diseases and therefore did not menstruate was not significantly different from the level characteristic of normally menstruating healthy women. Furthermore, the study of a number of rabbits of different ages showed that the males exhibit a significantly higher serum iron content than the females. The difference decreases with advancing age. As female rabbits do not menstruate, the possible effect of blood losses was eliminated, and it appeared logical to assume that sex hormones were responsible for the observed difference.

To test this hypothesis, Palmer administered to rabbits of both sexes relatively large doses of testosterone, progesterone, estrone and estradiol. Testosterone and progesterone were found to have no effect on serum iron levels. On the other hand, both forms of estrogen caused a rapid fall in plasma iron. This change was reversed when treatment was discontinued. *In vitro*, estrogen had no effect on the binding of iron to protein in rabbit, horse, or human plasma. On the other hand, estrogen was found to decrease the amount of iron released from respiring blood-free liver tissue in a liver plasma preparation. No similar effect could be demonstrated with spleen, kidney, lung or duodenal tissue.

G. R. Greenberg and M. M. Wintrobe (*J. Biol. Chem.* **165,** *397 (1946)*) have called the sum of the recently absorbed iron and that recently released during the breakdown of hemoglobin the "labile iron pool." Wallenius' and Palmer's reports throw some light on factors which do and factors which do not influence the release and transport of this labile iron on which tissues are dependent for the synthesis of iron-containing enzymes.

AUREOMYCIN, DIETARY PROTEINS AND THE GROWTH OF MICE

Aureomycin was the first antibiotic for which growth-promoting activity was suggested. The chick was used as the experimental animal (E. L. R. Stokstad and co-workers, *J. Biol. Chem.* **180,** *647 (1949)*; Stokstad and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* **73,** *523 (1950)*). The observation was extended to the pig (*Nutrition Reviews* **8,** *298 (1950)*) and turkey (*Ibid.* **9,** *359 (1951)*), as well as to the calf and rat. P. K. Vijayaraghavan, E. A. Murphy, and M. S. Dunn (*Arch. Biochem.* **36,** *127 (1952)*) have now extended the series of experimental animals, the growth of which can be stimulated by aureomycin, to include the laboratory mouse. They have also shown that this effect seems dependent on the biologic value of the dietary proteins.

Vijayaraghavan and co-workers placed groups of weanling rats of the A strain, males and females, on a number of "purified" and "natural" diets. The source of carbohydrate was either sucrose or corn meal. The protein was provided by casein, calf meal, soybean meal, peanut meal, or cottonseed meal. The diet of each experimental group was supplemented with 50 mg. per kilogram of diet of aureomycin hydrochloride. Addition of aureomycin to the diets containing casein, calf meal, or peanut meal as the source of protein was found to have no effect on the growth of the mice. By contrast, marked differences were found between growth rates on supplemented and unsupplemented diets based on soybean or cottonseed meal. Replacement of sucrose as a source of carbohydrate by corn meal did not affect the weight gain per gram of food.

The mechanism of this growth-stimulating activity of aureomycin or other antibiotics

is unknown. Possible explanations might be inhibition of toxin-producing organisms, selective inhibition favoring organisms producing vitamin B_{12} or other nutrients, or reduction of competition between host and flora for certain nutrients. A recent report (J. C. Van Meter, A. Spector, J. J. Oleson, and J. H. Williams, *Fed. Proc.* **11,** *301 (1952)*) also indicates that aureomycin may have marked effects on the metabolism of mammalian tissues and in particular on phosphorylation reactions. Vijayaraghavan and co-workers, recalling the fact that the majority of positive reports on the action of aureomycin on growth were obtained, as in their experiments with mice, with diets containing plant proteins (soybean and cottonseed), speculate that antibiotics may promote the development of a microflora which enhances the availability or the intestinal synthesis of critical amino acids. When proteins of high biologic value are provided, such as is the case with diets containing peanut protein, or the mixture of proteins found in calf meal or casein, these amino acids are not limiting factors for growth and the favorable effect of aureomycin cannot be observed. This conclusion appears to be a reasonable hypothesis, which could eventually be tested experimentally by adding the pure amino acids likely to be the least available to the diets showing the aureomycin effect, and seeing whether one or a mixture of these supplements does, in fact, cancel the aureomycin effect. The general interest in the mechanism of action of antibiotics on growth would certainly justify the testing of this interesting suggestion.

ENZYMATIC CONVERSION OF PHENYLALANINE TO TYROSINE

It was believed for some time that tyrosine was an essential amino acid. However, the early liver perfusion experiments of G. Embden and K. Baldes (*Biochem. Z.* **55,** *301 (1913)*) demonstrated that the addition of phenylalanine to the perfusion fluid resulted in formation of small amounts of tyrosine.

Another indication of the possibility of convertibility of phenylalanine into tyrosine could be found in the classic study by G. Medes (*Biochem. J.* **26,** *917 (1932)*) of a case of tyrosinosis. The case, an adult male, was characterized by excretion in the urine of the keto-acid which normally arises from the oxidative deamination of tyrosine, namely para-hydroxyphenylpyruvic acid. When phenylalanine was fed to this patient there was not only an increase in the excretion of this compound, but also the appearance of a sizable amount of tyrosine.

More direct information was provided by the work of R. S. Alcock (*Biochem. J.* **28,** *1174 (1934)*) and of M. Womack and W. C. Rose (*J. Biol. Chem.* **107,** *449 (1934)*), who demonstrated that tyrosine was not essential when enough phenylalanine was included in the diet. Further convincing proof for the *in vivo* conversion of phenylalanine to tyrosine was provided by A. R. Moss and R. Schoenheimer (*Ibid.* **135,** *415 (1940)*). These investigators prepared a synthetic phenylalanine in which the hydrogen of the benzene ring was replaced by deuterium. This phenylalanine was fed to rats for a ten-day period, and subsequently tyrosine was isolated from the tissue proteins. The tyrosine so obtained was found to contain a significant proportion of heavy hydrogen, indicating the direct conversion of dietary phenylalanine to tyrosine. On the other hand, while the existence of a conversion of phenylalanine to tyrosine *in vivo* was thus definitely established, information on the tissue catalysts responsible for this oxidation was meager. That the liver could effect

this conversion had been demonstrated by Embden and Baldes (*loc. cit.*). More recently, M. L. C. Bernheim and F. Bernheim (*Ibid.* **152,** *481* (*1944*)) had shown that surviving liver slices, on incubation with phenylalanine, produced an increased amount of phenolic material which showed many of the properties of tyrosine.

A recent report (S. Udenfriend and J. R. Cooper, *J. Biol. Chem.* **194,** *503* (*1952*)), representing considerable progress in the study of this problem, not only confirms the fact that liver slices and homogenates convert phenylalanine to tyrosine, but also describes the properties and requirements of a soluble system capable of catalyzing this reaction. The authors first tested the ability of slices or homogenates of various tissues to convert phenylalanine to tyrosine. Tyrosine, if formed, was determined by three methods: the study of the light absorption spectrum of the product obtained by coupling with a nitroso derivative; transformation, through the action of tyrosine decarboxylase, into tyramine; and paper chromatography.

It was found that livers of the rat, guinea pig, rabbit, dog, chicken and man convert phenylalanine to tyrosine. The enzyme system seems to be found only in the liver; at least, in the case of the rat no detectable activity could be found in the lung, kidney, brain or muscle. No measurable differences in rat liver enzyme could be correlated with age, sex, or strain. The authors also tested cell suspensions of three common microorganisms, *Streptococcus faecalis*, *Escherichia coli*, and yeast. They were found to be unable to carry out the conversion.

Udenfriend and Cooper went on to isolate the soluble system responsible for the conversion. The livers of decapitated rats were removed, homogenized, and centrifuged; the supernatant containing the active material was dialyzed and could be stored in the deep freeze. Further fractionation eliminated enzyme systems which attacked peptides and acetylated amino acids. Sufficient purification was achieved so that L-phenylalanine could be completely recovered as tyrosine and phenylalanine if the proper supplements were added. The test system utilized to study the requirements of the enzyme comprised the preparation, a phosphate buffer and nicotinamide. The system was found to require DPN or $DPNH_2$, with TPN as a less effective alternative. Oxygen was also required. No activity was found under strictly anaerobic conditions. Methylene blue or hydrogen peroxide could not substitute for molecular oxygen. The zone of optimal temperature was found to be between 25° and 35°C., the optimal pH 7. The enzyme content of the liver, as judged from the activity of the preparations, was found to be very high. Citrate buffers, cyanide, azide, and various phenylalanine analogues were found to inhibit the reaction. The enzyme was shown to be highly specific.

Interpreting their results, Udenfriend and Cooper note that in yeast and in *E. coli*, where independent pathways of synthesis have been demonstrated (B. D. Davis, *Experientia* **6,** *41* (*1950*); C. Gilvarg and K. Bloch, *Fed. Proc.* **10,** *189* (*1951*)), enzymatic conversion of phenylalanine to tyrosine does not seem to occur. In mammalian liver, the amount of activity found suggests that conversion to tyrosine is an important pathway of phenylalanine metabolism. That such a pathway is of essential importance had already been indicated by the study of oligophrenic phenylpyruvia, in which G. A. Jervis (*Proc. Soc. Exp. Biol. Med.* **75,** *83* (*1950*)) had shown that the biochemical lesion appeared to be the inability to convert phenylalanine to tyrosine, hence the accumulation of phenylalanine and its deamination product, phenylpyruvic acid.

The authors indicate that additional chemical work remains to be done. They point out evidence suggesting that two or more distinct enzymes may be involved in a chain using DPN and oxygen. Purification

and fractionation of the system has to be continued further. Equally important, from a physiologic standpoint, would be the elucidation of the factors which control, *in vivo*, the rate of transformation of phenylalanine to tyrosine.

This work takes on additional significance when it is recalled that tyrosine, besides its biologic importance per se, also represents one of the steps in the catabolism of phenylalanine. The enzyme system oxidizing phenylalanine to tyrosine initiates a chain of reactions on which light has recently been thrown. B. N. La Du and D. M. Greenberg (*J. Biol. Chem.* **190,** *245 (1951)*) showed that there is in the liver an active enzyme system which, when properly fortified, rapidly oxidizes tyrosine and other products of phenylalanine catabolism. The oxidative process probably involves a transamination yielding para-hydroxyphenylpyruvic acid, a decarboxylation yielding homogentisic acid, an opening of the benzene ring yielding fumarylacetoacetic acid which is then split into acetoacetic acid, one of the "ketone bodies" and fumaric acid, a member of the dicarboxylic acid cycle. Other investigators (*Nutrition Reviews* **10,** *117 (1952)*) have confirmed these findings.

More recently B. N. La Du (*Fed. Proc.* **11,** *244 (1952)*) has further defined some of the steps involved in the oxidation of tyrosine to acetoacetic acid. In particular, he showed that, provided alpha-ketoglutarate is present to permit the transamination step, the addition of ascorbic acid to an enzymatic extract inactivated by dialysis restores for a large part its activity. The stimulatory effect of ascorbic acid appears to be exerted on the final step following transamination, the transformation of phenylpyruvic acid to 2,5-dihydroxyphenylpyruvic acid. Indications exist that copper is also involved in the pathway of tyrosine oxidation.

The work of Udenfriend and Cooper thus provides one hitherto missing link in an enzymatic chain which now extends all the way from phenylalanine to carbon dioxide and water.

INSULIN AND COCARBOXYLASE IN DIABETES

In 1939, R. Goodhart and H. M. Sinclair (*J. Biol. Chem.* **132,** *11 (1940)*) conducted a systematic survey of blood cocarboxylase levels in a number of pathologic conditions. They found that, generally speaking, the amount of cocarboxylase in blood (determined by a chemical method) varied directly with the amount of total thiamine (determined by a biologic method) and also with the degree of saturation of the tissues with the vitamin. However, exceptions were found to this general rule. Some were related to cases where the blood cell count was greatly increased, for instance, in cases of polycythemia vera or myeloid leukemia. Patients who had recently received intensive therapy with vitamin B_1 over a very short period of time also exhibited high blood thiamine levels associated with a relatively low blood cocarboxylase content.

A more intriguing group of individuals showing such a discrepancy were 3 patients with diabetes, of whom 1 exhibited cardiac failure and 2 had a diabetic neuritis. One patient with a syndrome resembling Cushing's disease gave the same picture. Treatment with crystalline vitamin B_1 increased the levels of both blood cocarboxylase and blood thiamine, though the ratio of coenzyme to vitamin remained low.

Similar observations were recorded by a number of Italian authors, in particular V. Bisceglie (*Vitaminologia* **5,** *17 (1946)*). More recently, N. Siliprandi and his collaborators of the Institute of Biological Chemistry of the University of Rome have

conducted a number of experimental studies on the possible role of insulin in the synthesis of cocarboxylase. In a first series, Siliprandi (*Acta Vitaminol.* **4,** *259* (*1950*)) found that when alloxan-diabetic rats were injected with thiamine pyrophosphate, an immediate fall in the level of blood sugar, and a rise in the respiratory quotient and tissue glycogen ensued. Injections of large (20 mg. per kilogram of body weight) doses of thiamine led to similar effects, except in animals very severely diabetic. Injecting thiamine pyrophosphate in diabetic patients also led to a temporary fall in blood sugar and rise in respiratory quotient (Siliprandi and R. Traverso, *Farmaco* **5,** *655* (*1950*)).

These preliminary results led Siliprandi to the hypothesis that, in diabetes, thiamine could not be converted to cocarboxylase. This hypothesis was tested experimentally (D. Siliprandi and N. Siliprandi, *Nature* **168,** *422* (*1951*)). The cocarboxylase content of the liver of alloxan-diabetic animals was first determined, and found to be significantly lower than that of nondiabetic animals, the decrease seeming to be proportional to the severity of the diabetes. The effect of thiamine administration was next studied. Several groups of normal animals (15 to 22 animals per group) were injected with thiamine (20 mg. per kilogram of body weight) and sacrificed twenty minutes later. The cocarboxylase content of the liver was found to be increased on the average by 110 per cent. By contrast, similar groups of diabetic animals treated in the same fashion showed much smaller rises in liver cocarboxylase.

The respiratory quotients of a large group of moderately diabetic animals and of a similar group of severely diabetic animals were determined. The animals were then injected with thiamine (20 mg. per kilogram of body weight) and glucose (5 g. per kilogram of body weight). Sixty minutes later, the respiratory quotient was again determined. A few hours later another dose of thiamine was administered and liver cocarboxylase was determined under the same conditions as in the previous experiments. Results, as presented by the authors, were clear-cut. In a typical animal with moderate diabetes, the respiratory quotient rose from 0.74 before thiamine and glucose administration to 0.95 one hour after the double injection. The liver cocarboxylase level was found to be 13.74 mg. per gram of wet tissue.

By contrast, in a typical animal with severe diabetes, no rise in respiratory quotient was observed (0.65 before the thiamine-glucose treatment; 0.67 after the treatment). The liver cocarboxylase content was only 3.80 mg. per gram of wet tissue. The authors considered these results a proof of the fact that in alloxan diabetes phosphorylation of thiamine is impaired, and suggested that this might explain the utilization of pyruvic acid in diabetes.

In a further application of these results to human subjects, N. Siliprandi and F. Navazio (*Acta med. Scandinav.* **142,** *147* (*1952*)) determined the blood cocarboxylase in 13 normal individuals and 16 diabetic patients. In diabetic subjects, insulin treatment was suspended at least forty-eight hours before the start of the experiment. In all subjects the cocarboxylase content of blood was determined, 20 I. U. of insulin were administered intramuscularly, and cocarboxylase was then determined thirty minutes later. The average blood cocarboxylase content of the normal subjects was found to be 8.1 micrograms per 100 ml. of blood cocarboxylase with a standard deviation of ± 0.263 micrograms per 100 ml. The blood cocarboxylase content for the diabetic subjects before insulin administration was 5.0 ± 0.311 and after insulin administration it was 7.5 ± 0.579 micrograms per 100 ml. of blood. Furthermore, there was good correlation in the untreated diabetic patients between the degree of

hyperglycemia and the relative decrease in blood cocarboxylase. Conversely, the increase in cocarboxylase after administration of the fixed dose of insulin was inversely proportional to the severity of diabetes. Blood cocarboxylase was restored to normal levels in all except the most severe cases of diabetes. Again the authors conclude that the results strongly indicate that in diabetes mellitus insulin acts in the biosynthesis of cocarboxylase and, generally speaking, is necessary for the normal processes of thiamine phosphorylation.

It appears unnecessary to comment on these findings. If confirmed, they are obviously of great importance. It is to be hoped that other workers will want to repeat these experiments.

RELATION BETWEEN FOLACIN AND TISSUE ASCORBIC ACID

A number of experimental reports have suggested the existence of a close metabolic interrelationship between folacin and ascorbic acid. Earlier reports emphasized the relation of both ascorbic acid and folacin to the metabolism of aromatic amino acids. It will be recalled that human infants and guinea pigs excrete, when placed on ascorbic acid-deficient diets, abnormal products of the metabolism of phenylalanine and tyrosine. This excretion of abnormal phenolic compounds can be checked with ascorbic acid (*Nutrition Reviews* **5,** *173 (1947)*; **6,** *70 (1948)*; **10,** *92, 117 (1952)*). If folacin is given to scorbutic guinea pigs ingesting added quantities of tyrosine, the abnormal excretion is prevented, or if the administration of pteroylglutamates is started when the hydroxyphenylpyruvia is already established, this condition is eliminated (*Ibid.* **8,** *260 (1950)*; **10,** *92 (1952)*). Large doses of folacin also inhibit the tyrosyluria of scorbutic infants (J. E. Morris, E. R. Harpur, and A. Goldbloom, *J. Clin. Invest.* **29,** *325 (1950)*).

Recent evidence is believed to confirm the existence of this interrelationship between the two vitamins. It was shown (C. A. Nichol and A. D. Welch, *Proc. Soc. Exp. Biol. Med.* **74,** *52, 403 (1950)*) that ascorbic acid stimulates the conversion of folacin by rat liver slices to an essential growth factor for *Leuconostoc citrovorum*. J. N. Williams (*J. Biol. Chem.* **191,** *123 (1951)*; **192,** *81 (1951)*) reported that the lowered choline oxidase of the livers of rats fed aminopterin is stimulated *in vitro* by the addition of ascorbic acid. He has also shown (*Proc. Soc. Exp. Biol. Med.* **77,** *315 (1951)*) that the ascorbic acid content of the rat liver is decreased markedly by dietary aminopterin. Williams suggests, on the basis of these experiments, that folacin or the L. citrovorum factor (LCF) is involved in the maintenance of ascorbic acid levels in the rat.

In a following study, M. A. Schwartz and J. N. Williams (*J. Biol. Chem.* **194,** *711 (1952)*) went on to examine the possibility of reversing the depression of liver ascorbic acid levels caused by aminopterin by administering ascorbic acid or LCF to the aminopterin-treated animals. The authors reasoned that if such a reversal could be effected, this would constitute a demonstration of the direct involvement of folacin or LCF in the maintenance of ascorbic acid levels.

Accordingly, a number of adult male rats were given a basal ration containing aminopterin. Within ten days they showed a deficiency syndrome characterized by periocular and nasal deposition of porphyrin, diarrhea, and lack of spontaneous activity. The deficient animals were then separated into five groups; one receiving no supplement, two groups receiving graded daily supplements (60 micrograms and 500 micro-

grams) of folacin for three days, and two groups receiving graded daily supplements (60 micrograms and 500 micrograms) of LCF for three days. Two control groups had been kept on aminopterin-free diets, with one group receiving 200 micrograms of folacin per day for three days after ten days on the basal ration. At the end of the experimental period all animals were killed and liver ascorbic acid levels were determined.

It was found, as previously reported by Williams, that aminopterin markedly decreased the liver ascorbic acid levels. Folacin, when given to the normally fed animals, had no effect on the liver ascorbic acid levels. More important, folacin and LCF also failed to cause a definite rise in the liver ascorbic acid levels. The supplements also failed to enable the rats to recover from the folacin deficiency. Many of the animals died during the three-day supplementation period. The other animals still showed symptoms of folacin deficiency. The inability of high levels of folacin to reverse the toxic action of aminopterin has been reported previously (J. J. Oleson, B. L. Hutchings, and Y. SubbaRow, *J. Biol. Chem.* **175,** *359 (1948)*; I. T. Kline and R. I. Dorfman, *Proc. Soc. Exp. Biol. Med.* **76,** *203 (1951)*).

Schwartz and Williams do not draw any conclusion from these experiments on the interrelationship of folacin and ascorbic acid. They have warned the reader that if the reversal of ascorbic acid depressions due to aminopterin administration could not be effected by folacin or LCF, this should not rule out the possible role of folacin or LCF in the maintenance of tissue ascorbic acid, since the utilization of the pteroylglutamate compounds might be inhibited by dietary aminopterin.

It seems legitimate, however, to question not only the design of the experiment itself, but also the reasoning on which it is based. If the effect of aminopterin cannot be reversed by high doses of folacin, it is difficult to see why folacin administration should ever have been expected to restore to normal the biochemical mechanisms impaired by aminopterin. Furthermore, even if this reversal had been possible, one wonders whether it would have demonstrated the "direct involvement of folic acid (or LCF) in the maintenance of tissue ascorbic acid."

Clear evidence has been obtained of a decreased tissue ascorbic acid content in deficiencies of vitamin A (*Nutrition Reviews* **6,** *224 (1948)*), and of riboflavin and thiamine (*Ibid.* **7,** *52 (1949)*). Restriction of food alone has also been shown to exert some influence on ascorbic acid levels. When these deficiencies are eliminated, or when food intake ceases to be restricted, tissue ascorbic acid returns to higher levels. It is therefore questionable whether a decrease of levels of tissue ascorbic acid due to any deficiency, followed by a return to normal levels when the deficiency is eliminated, presents by itself the degree of specificity necessary for the demonstration of a close metabolic interrelationship. Conversely, it has been shown that administration of vitamin C delays the onset of vitamin A deficiency (J. Mayer, *Rev. canad. de biol.* **8,** *488 (1949)*) and of riboflavin and pantothenic acid deficiencies (F. S. Daft and K. Schwarz, *Fed. Proc.* **11,** *200 (1952)*). There again, no clear-cut proof exists that these effects are indicative of a specific interaction.

The early vitamin literature was replete with instances of "synergism" or "antagonism" between various vitamins. In most cases, these interrelationships have been shown to be either artifacts dependent on the use of pharmacologic, rather than physiologic doses, or coincidental consequences of long chains of unrelated biochemical events. Vitamin C, which is required in amounts much greater than other vitamins and which may have a nonspecific role in the maintenance of proper

oxidation-reduction conditions in tissues, has often been one of the postulated members of such interrelationships. One wonders if the postulated interrelationship of folacin and ascorbic acid really rests at this point on critical experimental evidence.

PHOTOSYNTHESIS AND COENZYMES

The fundamental, though indirect, part played by photosynthesis in the nutrition of higher animals enhances the importance of any research which throws light on this phytochemical reaction. It has been shown (H. G. Wood and C. H. Werkman, *J. Bact.* **30,** *332* (*1935*)) that unicellular organisms which require preformed organic nutrients can fix carbon dioxide. Later, by use of radioactive carbon, a similar fixation of carbon dioxide by tissue cells of higher animals (pigeon liver) was demonstrated (E. A. Evans and L. Slotin, *J. Biol. Chem.* **136,** *301* (*1940*)).

These observations suggested not only that there are other mechanisms than photosynthesis which permit the fixation of atmospheric carbon dioxide, but also that these mechanisms might be susceptible to study so that an experimental approach to the intricate photosynthetic process would be possible. Indeed, this hope seems to have been realized when it was observed (S. Ochoa, A. H. Mehler, and A. Kornberg, *J. Biol. Chem.* **174,** *979* (*1948*)) that cell-free enzyme preparations from animal and bacterial sources, acting in the presence of reduced pyridine nucleotide and manganous ions, catalyze the production of malate and isocitrate from pyruvic acid through the reductive fixation of carbon dioxide. In photosynthesis, there is a transfer of hydrogen from water, as the donor, to carbon dioxide as the ultimate acceptor; in the foregoing reaction the hydrogen donor is the reduced coenzyme.

In a recent report (W. Vishniac and S. Ochoa, *J. Biol. Chem.* **195,** *75* (*1952*)) evidence is brought forth supporting the view that chlorophyll preparations bring about a reduction of coenzymes I and II which permits the reductive fixation of carbon dioxide through the mediation of recognized enzyme systems. Green grana prepared from the chloroplasts of spinach leaves were suspended in a system containing pyruvate, coenzyme II, ATP, manganous chloride, potassium bicarbonate, potassium chloride, phosphate buffer (pH 7.0) and "malic enzyme" prepared from pigeon liver, the whole being kept under an atmosphere of nitrogen and carbon dioxide. After illumination of the system, the synthesis of malic acid could be demonstrated in as short a time as forty minutes; however, little if any synthesis of malate occurred if "malic enzyme" or the coenzyme was omitted or if the reaction flask remained in the dark. If alpha-ketoglutarate was used instead of pyruvate, isocitric acid was obtained as the final product. When the carbon dioxide was labeled with C^{14} and pyruvate was the substrate, the resulting L-malate contained the C^{14} in the beta-carboxyl group. In similar experiments with illumination when alpha-ketoglutaric acid was the substrate and the enzyme aconitase replaced the "malic enzyme," the end product was citrate.

That hydrogen acceptors other than carbon dioxide can be affected by the photochemical reaction was shown in the system containing the green grana, pyruvate, coenzyme I and lactic dehydrogenase in an atmosphere free of carbon dioxide; upon illumination lactate and oxygen were produced in the ratio of one mole of the former to one-half mole of the latter. In similar manner, malate was produced by the photochemical reduction of oxalacetate in the

presence of malic dehydrogenase. L-glutamic acid was obtained by photochemical reductive amination when the green grana were added to coenzyme I, ammonium chloride and glutamic dehydrogenase, and the system was illuminated in an atmosphere of nitrogen alone.

The photochemical reactions involving grana can be coupled with other natural enzyme systems capable of hydrogen transfer; extracts of *Escherichia coli* contain enzymes which, in the presence of molecular hydrogen, catalyze the reduction of fumarate to succinate. When green grana are added to an extract of the bacteria in an atmosphere of nitrogen only, succinate is formed from fumarate. This, together with variations of the same reaction, shows in another way that chlorophyll-bearing structures can produce hydrogen from water and that the further disposition of this hydrogen depends upon a variety of natural biologic chemical reactions. It is pointed out that the photochemical reduction of the pyridine nucleotides could also account for the formation of high energy phosphate bonds; by coupling with a phosphorylating system, the reduced coenzyme may be oxidized, thus yielding the energy-rich bonds and water.

The rate of photochemical reactions brought about by the green grana is very low compared to that in intact plants, a circumstance due possibly to lack of knowledge regarding all the factors involved in the transfer of hydrogen to the pyridine nucleotides. However, it appears that once the coenzymes have accepted the hydrogen produced either photosynthetically or chemosynthetically, further reactions such as the fixation of carbon dioxide, the reduction of intermediates, or formation of energy-rich phosphate bonds proceed as separate reactions even in the dark. The current evidence supports the view that the phenomenon of photosynthesis rests upon the activity of well-defined enzyme systems which are common to all cells.

THE UTILIZATION OF VITAMIN B_{12}

Present available evidence indicates that large doses of vitamin B_{12} are necessary, when given orally, to overcome symptoms of pernicious anemia in humans. In this disease there is an apparent impaired absorption of vitamin B_{12} from the gastrointestinal tract which can be overcome by the simultaneous administration of vitamin B_{12} and normal gastric juice (L. Berk *et al.*, *New Engl. J. Med.* **239,** *911 (1948)*).

B. F. Chow *et al.* (*Bull. Johns Hopkins Hosp.* **87,** *156 (1950)*) found that the oral administration of massive doses (one hundred or more times the normal therapeutic dose) caused no appearance of this vitamin in the urine. On the other hand, the major portion of parenterally administered vitamin B_{12} was recovered in the urine. From these data it was indicated that vitamin B_{12} is apparently not absorbed in any large quantities in normal individuals.

Recent evidence of the absorption of vitamin B_{12} following oral administration in experimental animals has been reported from three different laboratories. R. Yamamoto, C. Barrows, C. Lang, and B. F. Chow (*J. Nutrition* **45,** *507 (1951)*) studied the urine and blood levels of vitamin B_{12} following oral and subcutaneous administration of the vitamin in dogs. The absorption of the vitamin following oral administration was substantially proved on the basis of the blood levels. When 900 micrograms of vitamin B_{12} per kilogram body weight were administered orally the vitamin activity in plasma increased from an initial zero level to 9.2 millimicrograms per milliliter after two hours. The amount excreted via the

urine in twenty-four hours was only 1.5 per cent of the dose administered. No plasma vitamin B_{12} was detectable, however, after the oral administration of 150 micrograms of the vitamin, whereas 6 micrograms per kilogram, intravenously administered, produced plasma levels of 11 millimicrograms per milliliter of plasma within thirty minutes after administration. Approximately 90 per cent of the intravenously administered vitamin B_{12} was recovered in the urine.

Evidence for the absorption of orally administered vitamin B_{12} by the rat was indicated by the studies of M. L. Meyer, H. T. Thompson, and C. A. Elvehjem (*J. Nutrition* **45,** *551 (1951)*). These workers demonstrated that the vitamin B_{12} content of rat's milk varied with the diet fed. A vitamin B_{12} supplement of 10 micrograms per 100 g. of diet increased the vitamin B_{12} content of the rat's milk to 139 millimicrograms per milliliter, compared with a level of 4.9 millimicrograms on the unsupplemented corn-soybean meal-alfalfa basal diet. Fish solubles added to the basal diet at a level to supply about 1.5 micrograms of vitamin B_{12} per 100 g. of diet increased the vitamin B_{12} content of the rat's milk to 26.9 millimicrograms per milliliter.

These workers noted that rat's milk is a rich vitamin B_{12} source compared with the values reported for milk in other species. Sow's milk, for example, has been reported to contain 1.05; horse milk, 0.02; and human milk, 0.41 micrograms per liter. It was suggested that the high level of protein in rat milk (11.8 per cent) may increase the demand for vitamin B_{12} by suckling young in this species.

The results of these two groups of investigators leave little doubt that orally administered vitamin B_{12} is absorbed, and the results also indicate that the level of vitamin B_{12} in the diet has a marked effect on the level of this vitamin in the milk of rats.

The need for considering the influence of the diet as a whole was strikingly emphasized by the study of Meyer and associates. Roast beef as a protein source in the diet invariably resulted in relatively high levels of vitamin B_{12} in the rat's milk, ranging from approximately 15 to 25 millimicrograms per milliliter of milk. On the other hand, when roast pork served as the dietary protein source, the vitamin B_{12} content of the rat's milk varied greatly and was correlated with the reproductive performance of the females. Only 4.5 millimicrograms of vitamin B_{12} per milliliter of milk were found when the females exhibited lactation failure, whereas levels in the range noted with roast beef diets were found when lactation behavior of the females was normal. It had been noted earlier (L. M. Henderson, B. S. Schweigert, A. K. Mozingo, and C. A. Elvehjem, *J. Nutrition* **36,** *479 (1948)*) that roast pork diets frequently failed to support normal reproduction and lactation in rats. This unexplained failure of normal reproductive performance could be corrected by substituting roast beef or casein for roast pork in the diet. The recent study suggests that pork may contain some factor or factors that somehow interfere with the normal utilization of vitamin B_{12} as evidenced by extreme variations in concentration of this vitamin in the milk.

S. Levey and J. M. Orten (*J. Nutrition* **45,** *487 (1951)*) investigated the relation of vitamin B_{12} to the production of polycythemia by cobalt in rats. It was presumed that the mechanism of cobalt polycythemia induction might involve increased vitamin B_{12} formation, a cobalt-containing vitamin that is now known to have a profound effect on the formation of red blood cells. In their studies they noted that the injection of vitamin B_{12} (3 to 10 micrograms per rat daily) increased the liver stores of this vitamin from a control level of 0.121 micrograms per gram to 0.155 micrograms per gram. Cobalt injections (0.5 milligrams per

rat daily as cobaltous sulfate) produced the expected increase in red blood cells, but failed to influence liver vitamin B_{12} storage.

Untreated control animals had fecal vitamin B_{12} values of 3.3 micrograms per gram. Cobalt injection caused some increase in fecal vitamin B_{12} content (5.2 micrograms per gram) whereas injection of vitamin B_{12} decreased the fecal content of this vitamin (1.65 micrograms per gram). Whether or not the differences were significant was not established.

From these results it was concluded that the polycythemia-inducing action of cobalt cannot be explained on the basis of an increased formation of vitamin B_{12} in the rat. Although these studies establish the fact that orally administered vitamin B_{12} is absorbed, the efficiency of this process has not been established. Most of the present evidence indicates that the absorption varies considerably, and the results of Meyer and associates indicate that the diet used can apparently influence the effectiveness of orally administered vitamin B_{12} considerably.

PANTOTHENIC ACID DEFICIENCY AND ADRENAL INSUFFICIENCY

That the relation between pantothenic acid and structural or functional integrity of the adrenal cortex is a close one has been often suggested (*Nutrition Reviews* **8,** *25 (1950)*). Pantothenic acid deficiency has been shown to cause atrophy of the adrenal cortex with lipid depletion (A. F. Morgan and H. D. Simms, *Science* **89,** *565 (1939)*; *J. Nutrition* **19,** *233 (1940)*; H. W. Deane and J. M. McKibbin, *Endocrinology* **38,** *385 (1946)*). In its acute form, it causes adrenal hemorrhage and necrosis (F. S. Daft and W. H. Sebrell, *Pub. Health Reports* **54,** *2247 (1939)*; A. A. Nelson, *Ibid.* **54,** *2250 (1939)*). This symptom can be cured by administration of pantothenic acid (L. L. Ashburn, *Ibid.* **55,** *1337 (1940)*).

However, if this relation exists, it is not a simple one, as many of the facts uncovered seem to be almost in contradiction with one another. Morgan and Simms (*J. Nutrition, loc. cit.*) found that the achromotrichia (graying) of rats fed diets free of "filtrate factor" could be at least partly reversed by extracts of the thyroid and of the adrenal cortex. On the other hand, E. P. Ralli and I. Graef (*Endocrinology* **32,** *1 (1943)*; **37,** *252 (1945)*) found that adrenalectomy prevented gray hair in pantothenic acid-deficient rats and caused reversal of achromatrichia due to lack of this vitamin. Two reports (Ralli, *Ibid.* **39,** *225 (1946)*; M. E. Dumm and Ralli, *Ibid.* **43,** *283 (1948)*) indicate that adrenalectomy increases requirements for pantothenic acid. On the other hand, adrenalectomy was found not to modify the proportion of administered pantothenic acid which is excreted in the urine (Dumm and Ralli, *Ibid.* **45,** *188 (1949)*).

A recent study by L. S. Hurley and A. F. Morgan (*J. Biol. Chem.* **195,** *583 (1952)*) is based on the reasoning that if it is true, as suggested from the histologic and physiologic evidence at hand, that pantothenic acid deficiency imposes a continuous stress on the adrenal cortex, then, according to H. Selye's "alarm reaction" model (*J. Clin. Endocrinol.* **6,** *117 (1946)*), exhaustion of the adrenals should occur, and the deficient animals should act as though adrenalectomized if exposed to a second stress. Hurley and Morgan chose as the cause of a second stress anoxic anemia, decreasing the oxygen tension to that prevailing at 20,000 feet of altitude. The responses of normal and of pantothenic acid-deficient animals were compared with respect to liver glycogen and blood glucose, adrenal ascorbic acid concentration and adrenal weights. Some of

the deficient animals were also given one dose of pantothenic acid, adrenal cortical extracts, and desoxycorticosterone prior to the reduction in oxygen tension. Inanition controls were kept.

It was found that, while the normal rats increased their liver glycogen by 600 to 700 per cent, under reduced tension, the pantothenic acid-deficient rats showed no rise in liver glycogen during the stress period of anoxia. Similar observations were made on the blood sugar values. The deficient animals also showed lower ascorbic acid values and greater adrenal weights than the controls. Administration of one dose of pantothenic acid did not produce a very marked rise of glycogen or blood glucose. By contrast, administration of adrenal cortical extract (but not of desoxycorticosterone), enabled the deficient rats to raise their liver glycogen and blood sugar under anoxia as well as the control group.

The inanition controls had high levels of liver glycogen before anoxia. The levels rose further when the oxygen tension was reduced, thus showing that inanition alone could not account for the failure of the deficient animals to react normally.

The authors conclude from these results that it is probably a functional deficiency of adrenal cortical hormones which produces the failure of carbohydrate metabolism under stress. The fact that their administration can instantly restore this mechanism while the effect of pantothenic acid is delayed specifically points to the adrenal cortex as the site of the defect. By contrast, it has been shown (M. E. Wickson and A. F. Morgan, *J. Biol. Chem.* **162,** *209 (1946)*) that, while riboflavin-deficient rats also fail to raise their carbohydrate levels under anoxic stress, this ability is fully restored when one moderate dose of riboflavin is injected just before the test. In that case, immediate repair of the carbohydrate mechanism is therefore possible, while with pantothenic acid it is delayed, presumably because of the need for cortical hormone synthesis.

Demonstration of the absence or presence of a substance in the organism through observation of biochemical responses is always difficult. Pathologic signs like adrenal enlargement or drop in adrenal ascorbic acid are completely nonspecific. Alternative explanations to such coincidental happenings, although improbable, are by no means impossible. It is regrettable that certain more specific observations were not made or recorded in the experiments presented by Hurley and Morgan. One wonders, for example, what the eosinophil response to anoxia was in normal and deficient animals. A drop in eosinophil count is a more informative index of adrenal activity than a rise in liver glycogen. Administration of corticotrophin would have yielded necessary information on the ability of the adrenals of the deficient animals to secrete cortical hormones. Administration of epinephrine would have shown whether the pituitary could secrete ACTH. The authors have at present no more ground to implicate the adrenals that one could have to implicate the pituitary for this possible failure of cortical function.

This study again contributes a suggestion that there is a close link between pantothenic acid and adrenal function, but a clear-cut answer to the problem is still needed.

NOTES

Letter to the Editor

Dear Sir:

The exceptionally favorable review given to the work of R. Zetterström and M. Ljunggren on the enzymatic action of vitamin D (*Nutrition Reviews* **10,** *25* (*1952*)) does not seem to be in keeping with the standards of careful appraisal usually maintained by *Nutrition Reviews*. In the introductory part of this review, the reader's attention is called to " . . . the fact that other workers had been able to study the effects of alpha-tocopherol and of certain estrogenic hormones in the aqueous phase by preparing the phosphorylated derivatives . . ." However, it should be recalled that the observed *in vitro* effects of alpha-tocopherol phosphate are regarded by most workers in the field as being nonspecific and related to the properties of this substance as an anion with large nonpolar groups (*e. g., Ibid.* **8,** *13* (*1950*); M. Rabinovitz and P. D. Boyer, *J. Biol. Chem.* **183,** *111* (*1950*); S. R. Ames and H. A. Risley, *Ann. N. Y. Acad. Sci.* **52,** *149* (*1949*); Boyer, Rabinovitz, and E. Liebe, *Ibid., p. 188;* C. van der Meer and H. T. M. Nieuwerkerk, *Biochim. et biophys. acta* **7,** *263* (*1951*)). With the experiences from studies of alpha-tocopherol phosphate as a background, the biologic significance of *in vitro* studies with phosphorylated derivatives of fat-soluble vitamins is clearly open to question, in particular when evidence of any specificity of the action has not been published.

The experiments of Zetterström may be of definite biologic significance. However, the results reported to date are open to logical alternate interpretations. Most important is that trials on specificity, such as measurements with other sterol phosphates, are not reported. In addition, the reported stimulation of oxygen uptake of mitochondria could readily result from nonspecific dissociation of oxidation from net phosphate esterification.

While Zetterström's concept of the action of phosphorylated vitamin D_2 presents an attractive hypothesis, the supporting evidence presented thus far appears to be insufficient to warrant overenthusiastic interpretation of the data. A far-reaching integration of fragmentary evidence can readily lead to establishment of erroneous concepts in the literature.

PAUL D. BOYER
Division of Agricultural Biochemistry
University of Minnesota
St. Paul 1, Minn.

Recent Nutrition Book

Four Thousand Million Mouths. Scientific Humanism and the Shadow of World Hunger.

Edited by F. Le Gros Clark and N. W. Pirie. Oxford University Press, 1951. Pp. xii + 222. Price: 12s. 6d.

CLINICAL NUTRITION BRIEFS

"The increased incidence of degenerative diseases such as hypertension, coronary disease, and diabetes among the overweight has been firmly established. With a good deal of justification caloric overnutrition has been termed the most prevalent American nutritional disease." (*Nutrition Reviews* **10,** *109* (*1952*), Management of Overweight.)

"These studies have suggested that pernicious anemia may occur in children more frequently than has been supposed and that histamine-refractory achlorhydria may not be essential to the diagnosis if evidence for the absence of intrinsic factor is obtained." (*Nutrition Reviews* **10,** *113* (*1952*), Pernicious Anemia in Childhood.)

"From a nutritional standpoint, it is possible to treat ascites and edema accumulation in patients with cirrhosis of the liver effectively with a low sodium diet. Moreover, these patients seem to tolerate a low sodium diet better than patients with heart failure or particularly those with hypertension." (*Nutrition Reviews* **10,** *130* (*1952*), Metabolism of Water and Electrolytes in Patients with Liver Disease.)

"The authors describe another patient who required emergency surgery for intestinal obstruction while under anticoagulant therapy. Again vitamin K_1 emulsion given intravenously was associated with a rapid rise of prothrombin activity to manifestly safer levels . . . It may be anticipated that emulsions of vitamin K_1 will be just as effective or more so in the treatment of hypoprothrombinemia induced by some of the newer antiprothrombin drugs." (*Nutrition Reviews* **10,** *139* (*1952*), Vitamin K_1 Emulsions in Anticoagulant Therapy.)

"Since vitamin B_{12} appears to have no stimulating effect on the growth of premature infants, one may be justified in assuming that these infants are adequately supplied with this vitamin either by tissue reserves or from their food." (*Nutrition Reviews* **10,** *146* (*1952*), Vitamin B_{12} and Growth of Premature Infants.)

"In view of the paucity of signs and symptoms exhibited by their subjects despite an estimated loss of about 5 per cent of the body potassium, Black and Milne doubt whether potassium deficiency per se is responsible for many of the clinical symptoms and signs commonly ascribed to hypokaliemia." (*Nutrition Reviews* **10,** *163* (*1952*), Experimental Potassium Depletion in Man.)

"The data can be interpreted as showing simple iodine deficiency among those individuals with high radioactive iodine uptakes. The authors attribute the normal and low uptakes of some of their goitrous patients to the sporadic medicinal use of iodine." (*Nutrition Reviews* **10,** *167* (*1952*), Thyroid Uptake of Iodine in Goiter.)

"This series of reports indicates that consistently similar improvement in the nutrition of patients with nontropical sprue is obtainable by the use of ACTH or cortisone. The temporary nature of the remissions induced, however, indicates that the fundamental defect in this disease is probably not altered by these hormones." (*Nutrition Reviews* **10,** *169* (*1952*), Cortisone and ACTH in Nontropical Sprue.)